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THE VALUE OF THE WASSERMANN REACTION IN OBSTETRICS, BASED UPON THE STUDY OF 4547 CONSECUTIVE CASES*

By J. Whitridge Williams

I have always been interested in the relations between syphilis and obstetrics. This interest originated in connection with the microscopic examination of the placenta, which has been a routine procedure since the beginning of our service, with the result that we have now available the records of some twenty thousand such examinations. That study soon taught me that lesions frequently occur in the placenta which are characteristic of syphilis, and which enable one to detect the disease in many cases in which its existence is otherwise unsuspected.

When the Wassermann reaction was first described, I welcomed it as an additional aid in diagnosis, and immediately began to compare the results obtained by its means with those following the microscopic examination of the placenta. For a number of years, however, I employed it only when something in the history of the patient suggested the possible existence of syphilis. Although my investigations had shown that syphilis was a frequent complication of pregnancy and was one of the common causes of fœtal death, I did not appreciate its full significance in obstetrical work until 1915. At that time I studied the fœtal and infantile deaths in a series of 10,000 consecutive deliveries, and in my presidential address before the American Association for the Prevention of Infantile Mortality, I stated that syphilis was the most important single cause and constituted the etiological factor in 26 per cent of the deaths occurring in my service between the end of the seventh month of pregnancy and the two weeks immediately following delivery.

As the result of that study, I concluded that the most immediately fruitful field for prenatal work lay in the earliest possible recognition of the existence of syphilis and its intensive treatment during pregnancy, and that this could best be accomplished by making a Wassermann test upon every patient entering the service. Consequently, from April, 1916, to the present time the routine procedure is to withdraw a specimen of blood from every patient at her first visit to the dispensary and, if a positive Wassermann is obtained, to subject her to intensive treatment in the syphilitic department of the hospital. Furthermore, in the hope of increasing our knowledge concerning the incidence of the disease, as well as its clinical significance for the mother and child, a Wassermann is likewise made from a sample of fœtal blood obtained from the umbilical cord immediately after delivery. Likewise, every placenta is weighed and described macroscopically, after which portions of it are hardened, cut, stained and subjected to microscopic examination. Finally, whenever the

*Read before the American Gynecological Society, May 24, 1920.

child is born dead or dies within the first two weeks of the puerperium, every effort is made to secure an autopsy, at which particular attention is paid to the detection of syphilitic lesions, and a positive anatomical diagnosis is not made unless spirochetes can be demonstrated in the fœtal organs.

The present work is based upon the study along these lines of four thousand women and their children who were delivered in the service, out of 4547 admissions, between April, 1916, and December 31, 1919. The patients were almost equally divided between whites and blacks, though the latter slightly predominated—1839 and 2161, respectively.

On this occasion I shall refer incidentally to the part played by syphilis in the causation of fœtal death, then I shall consider more fully the significance of the maternal and fœtal Wassermann, after that I shall consider briefly the value of the microscopic examination of the placenta in the detection of syphilis, and finally say a few words concerning the status of Colles' law.

I am able to make the remarks concerning syphilis as a cause of fœtal death comparatively brief, for the reason that the subject was considered in detail in my article on "The Significance of Syphilis in Prenatal Care and in the Causation of Fœtal Death," which appeared in the BULLETIN of The Johns Hopkins Hospital, May, 1920.

That article was based upon the study of 302 fœtal deaths—99 whites and 203 blacks—which occurred in the 4000 deliveries under consideration. It showed that syphilis was the most important single cause of death, and that it was responsible for 34.4 per cent of the total number. These figures did not include the children discharged alive with hereditary syphilis, nor those in whom the disease developed later. Furthermore, it was shown that syphilis was responsible for more than twice as many deaths as the next most important cause, namely dystocia, as well as for nearly as many deaths as the next three most common causes combined, namely dystocia, toxæmia and prematurity. My figures indicated that these three causes were responsible for 37 per cent of the deaths as compared with 34.4 per cent for syphilis. At the same time, I pointed out the great difference in the incidence of the disease in the two races; syphilis being responsible for 12 of the 99 white, as contrasted with 92 of the 203 black deaths, an incidence of 1 to 8, and 1 to 2, respectively.

After these preliminary remarks, I shall pass on to the study of the significance of the Wassermann reaction in pregnant women. In the 4000 women delivered during the period under consideration, 449, or 11.2 per cent, presented a positive reaction during pregnancy. Its incidence was much greater in the black than in the white women, being 16.29 per cent and 2.48 per cent, respectively. In other words a positive Wassermann was noted in every sixth colored woman, as compared with every fortieth white woman.

It is comparatively easy to collect such gross figures, but when we attempt to determine their significance, we immediately fall into very great difficulty, as we are brought face to face with a number of conditions which we cannot at present satisfactorily explain.

Chart I gives an idea of the complexity of the problem. The 449 cases included in it are arranged in three great groups, according as the patients were not treated at all, were treated imperfectly, or were well treated prior to delivery. In each group there are a number of subdivisions, amounting to 24 in all, which indicate as far as possible the condition of the child, the microscopical findings in the placenta and the results at autopsy. By way of explanation, it may be said that the treatment was regarded as efficient only when the patient had been given a course of five or six injections of salvarsan followed by mecurial treatment, which resulted in the permanent disappearance of the positive Wassermann. Under inefficient treatment were included such patients as had received one or more injections of salvarsan, but in whom, for one reason or another, the course of treatment had not been completed; while, of course, no explanation is required for the patients who received no treatment, except in so far as failure to give it is concerned. In most cases in this category lack of treatment was due to the fact that the women were registered late in pregnancy, and fell in labor before it could be instituted; while occasionally it was due to the fact that the patients did not return for treatment after having been directed to do so.

With these figures before us, it may be asked, What is the significance of a positive Wassermann reaction occurring in a pregnant woman? Does it inevitably mean that she has syphilis, and will she transmit the disease to the child? To the first question, I am not prepared to give a conclusive answer, although I shall have more to say concerning it in the section devoted to Colles' law; but concerning the second question, our observations permit a definite statement to the effect that the existence of a positive Wassermann on the part of the mother does not necessarily mean that her child will develop syphilis.

This is clearly demonstrated by the consideration of the untreated cases included in Chart I. Our figures show that 87 of the 169 children concerned (A and C), or 51.5 per cent, were discharged in good condition, or were born dead or died during the puerperium, but presented no signs of syphilis at autopsy. Moreover, in all of the 87 cases the microscopic examination of the placenta was negative. On the other hand, 57 children presented either clinical or anatomical evidences of syphilis (B, E, G and I). In 19 other cases the placenta was syphilitic, and thus raised the presumption that the child was likewise suffering from the disease. Closer study, however, shows that such is not inevitably the case; as in four instances (H) the children were discharged in good condition, while in two others (J) they died, but showed no signs of syphilis at autopsy. In 13 cases (D) the children died, but did not come to autopsy, so that a positive statement as to the cause of death cannot be made, although the presumption is that it was due to syphilis. Finally, in six other cases (F) the placenta was normal, but the dead children were not autopsied. In this group, likewise, a positive answer cannot be given, although the presumption is that syphilis was not the cause of death. In other words, of the children born to 169 women

presenting a positive Wassermann during pregnancy, but who were not subjected to treatment of any sort, 51.5 per cent showed no evidence of syphilis, 33.7 per cent had definite syphilis, while in 14.7 per cent the results were doubtful. Consequently, it appears conservative to assume that the evidence at our disposal indicates that less than one-half of such women, and possibly even a smaller number, will have syphilitic children.

Chart I also affords a striking demonstration of the beneficial effect upon the child of treatment administered to the mother during pregnancy. Thus, while 51.5 per cent of the children of the 169 women who were not treated showed no signs of syphilis, 66.8 per cent were free from the disease in the 102 women who were inefficiently treated, as contrasted with 93.6 per cent in the 178 women who were well treated. Or, to express it in another way, the incidence of syphilis was 48.5, 33.2, and 6.7 per cent in the three groups respectively. Such figures are extremely encouraging and might tempt one to conclude that ideal prenatal care might lead to the total abolition of syphilis in the child. Such a conclusion, however, is too roseate, and as the sequel will show is not justified.

Chart II gives a totally different aspect to the question. It is based upon the study of 109 other women in the series who presented a negative Wassermann during pregnancy, but in whose history some mention of syphilis was made. Roughly speaking, these women may be classified in four categories: (a) Those who had presented a positive Wassermann in a previous pregnancy, but who had been treated and apparently cured; (b) those in whom autopsy upon the child conclusively demonstrated the existence of syphilis; (c) those presenting a positive placenta and an apparently normal child; and (d) cases in which the paternal Wassermann was positive. Upon analyzing the cases included in this chart, we find that 44 women had been effectively treated in a previous pregnancy, and may be regarded as having been cured of syphilis, leaving 65 for consideration. These 65 women had 54 dead and 11 live children. Forty-three of the dead children came to autopsy and 11 did not; 40 of the former were definitely syphilitic, while 3 were not. Of the 11 children, which were not autopsied, 7 (D) had positive placentæ and presumably were syphilitic, while in 4 (F) the placenta was normal and the mother had been treated in a previous pregnancy. Of the 11 children which were born alive, 3 presented characteristic lesions of hereditary syphilis (I and L); while 8 were discharged in good condition (E, G, and O). Concerning the three cases in subdivision G, more will be said in the section dealing with Colles' law.

In other words, of the 65 children under consideration, 43 were definitely syphilitic; 11 others died, but were not autopsied; 3 gave negative results at autopsy; and 8 were discharged in good condition.

What does this mean? At first glance it would appear to invalidate almost entirely the value of the routine Wassermann in obstetrics, but a little further consideration indicates that so pessimistic a conclusion is not justified. All that we can say positively is that 43 syphilitic children were born to 65 women who presented a negative Wassermann.

What this means serologically it is impossible to state at this time, but practically it means that in our material about one baby in 100 (43 out of 4000) will have syphilis even when the maternal Wassermann is negative, and consequently one is not justified in claiming that the most ideal prenatal care can entirely eradicate the disease as a cause of fœtal death. Of course, in practice the results will not be quite so bad as here indicated, for the reason that the condition would probably be recognized after the birth of the first syphilitic child, when the mother would be properly treated with a reasonable prospect that future children would be exempt from the disease.

The practical bearing of this aspect of the problem may perhaps be elucidated by a little calculation. For example, if it is assumed that 11 per cent of our women have a positive Wassermann, and that without treatment one-half of their children would be syphilitic, we should expect 55 syphilitic children in every 1000, plus 10 others (1 per cent), which would be born of women with a negative Wassermann, or a total of 65 per 1000. Consequently, our figures indicate that even though routine Wassermann tests were made early upon all pregnant women and efficient treatment instituted at once, only five-sixths ideal results would be obtained. Of course this would apply only to the first delivery in the service, as in the women with negative Wassermanns the existence of syphilis would be detected after the birth of the first child, when treatment would be immediately instituted and be followed by excellent results in the future.

Generally speaking, I feel justified in concluding that such a result should not discourage us, for if we were able to reduce the fœtal mortality from syphilis by five-sixths, its eventual incidence would scarcely exceed 1 per cent, and it would be converted from the most common cause of fœtal death into an infrequent one.

Turning to the consideration of the significance of the fœtal Wassermann at the time of delivery, our material shows that a positive result was obtained in 38 of the 4000 observations, approximately 1 per cent. This means that only a small fraction of the children born of mothers with a positive Wassermann present such a reaction. It should, however, be remembered that macerated children are not available for the test, as their blood is already "laked."

In order to arrive at a conclusion as to the value of such investigations, we have attempted to determine the fate of the 38 children concerned. For this purpose, they were visited at their homes, subjected to a careful physical examination, and a sample of blood was removed in order that the Wassermann might be repeated. As three years had elapsed since the oldest cases had been discharged, it is not surprising that nine of them could not be located, thus leaving 29 available for consideration. Fourteen of the children died within the first month, mostly in the service, and in 12 of them syphilis was demonstrated. Five of the surviving children developed clinical syphilis later, while 10 presented no clinical signs of

TOTAL NUMBER OF CASES (449).
12⅓ inches
169 Cases, No Treatment.
68 No treatment, placenta and child normal.
40 No treatment, placenta syphilis, child syphilis.
18 No treatment, placenta normal, autopsy negative.
13 No treatment, placenta syphilis, child dead, no autopsy.
9 No treatment, placenta normal, autopsy syphilis.
6 No treatment, placenta normal, child dead, no autopsy.
4 No treatment, placenta normal, child clinical syphilis.
4 No treatment, placenta syphilis, child living and well.
4 No treatment, placenta syphilis, child clinical syphilis.
2 No treatment, placenta syphilis, autopsy negative.
102 Cases, Inefficient Treatment.
62 Treatment inefficient, placenta and child normal.
10 Treatment inefficient, placenta syphilis, autopsy syphilis.
9 Treatment inefficient, placenta syphilis, child dead, no autopsy.
6 Treatment inefficient, placenta normal, child clinical syphilis.
5 Treatment inefficient, placenta syphilis, autopsy negative.
4 Treatment inefficient, placenta normal, child dead, no autopsy.
3 Treatment inefficient, placenta normal, autopsy negative.
2 Treatment inefficient, placenta normal, child clinical syphilis.
1 Treatment inefficient, placenta syphilis, child living and well.
178 Cases, Good Treatment.
166 Treatment good, placenta normal, child normal.
6 Treatment good, Wass. still +, placenta normal, child normal.
3 Treatment good, placenta normal, child syphilis.
2 Treatment good, placenta syphilis, child normal.
1 Treatment good, placenta syphilis, child dead, no autopsy.
A B C D E F G H I J K L M N O P Q R S T U V W X

Chart I.—Graphic analysis of results in 449 women presenting a positive Wassermann (1/36 inch to case).

169 Patients with no treatment had 48.5% syphilitic children.
102 Patients with inefficient treatment had 39.2% syphilitic children.
179 Patients with efficient treatment had 6.7% syphilitic children.

TOTAL NUMBER OF CASES (109).
44 Following treatment in previous pregnancy, placenta and child normal.
24 No treatment, placenta syphilis, autopsy syphilis.
10 No treatment, placenta normal, autopsy syphilis.
7 No treatment, placenta syphilis, child dead, no autopsy.
4 No treatment, placenta syphilis, child normal.
4 Following treatment in previous preg., placenta normal, child dead, no autopsy.
3 Following positive Wass. without treatment, placenta and child normal.
2 No treatment, placenta syphilis, autopsy negative.
2 No treatment, placenta normal, child clinical syphilis.
2 Treatment inefficient, placenta normal, autopsy syphilis.
2 Treatment in previous pregnancy, placenta syphilis, autopsy syphilis
1 No treatment, placenta syphilis, child clinically syphilis.
1 Paternal Wass. +, placenta syphilis, autopsy syphilis.
1 Paternal Wass. +, placenta normal, autopsy syphilis.
1 Paternal Wass. +, placenta normal, child normal.
1 Treatment in previous pregnancy, placenta normal, autopsy negative.
A B C D E F G H I J K L M N O P

Chart II.—Graphic analysis of 109 cases in which the maternal Wassermann was negative, but in which some mention of syphilis was made in the history (1/18 inch to case).

B 24	I 2	D 7	E 4	H 2
C 10	L 1	F 4	G 3	P 1
J 2			O 1	
K 2				
M 1				
N 1				
40	3	11	8	3
Positive autopsy	Clinical syphilis	Child dead no autopsy	Child normal	Autopsy negative

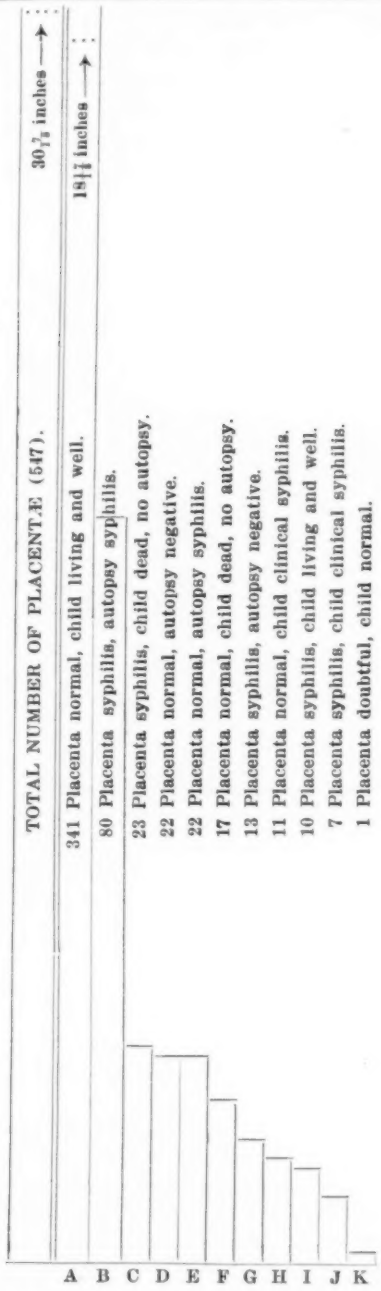
TOTAL NUMBER OF PLACENTÆ (547). 30 7/8 inches
341 Placenta normal, child living and well. 18 1/4 inches
80 Placenta syphilis, autopsy syphilis.
23 Placenta syphilis, child dead, no autopsy.
22 Placenta normal, autopsy negative.
22 Placenta normal, autopsy syphilis.
17 Placenta normal, child dead, no autopsy.
13 Placenta syphilis, autopsy negative.
11 Placenta normal, child clinical syphilis.
10 Placenta syphilis, child living and well.
7 Placenta syphilis, child clinical syphilis.
1 Placenta doubtful, child normal.
A B C D E F G H I J K

Chart III.—Graphic analysis of results of microscopic examination of 547 placentæ from patients included in Charts I and II (1/18 inch to case). The difference between 558 and 547 is due to the fact that the sections from 11 placentæ were lost, and consequently could not be verified.

the disease. In the latter a Wassermann repeated months or years after the original gave positive results in five children, while it was negative in the other five.

In other words, out of the 29 children who presented a positive Wassermann at birth, 17 developed definite evidences of syphilis, 5 showed no clinical signs, but continued to have a positive Wassermann, while 7 showed no signs in association with a negative Wassermann. Accepting the positive Wassermann as evidence of the existence of syphilis in the child, it appears that the primary reaction corresponded with the clinical and anatomical findings in 76 per cent of the cases.

At first glance these figures would seem to indicate that the fœtal Wassermann is of considerable prognostic value, and indicates that the great majority of children presenting it either have syphilis or will develop it later. The validity of such a conclusion, however, is put in serious doubt when an attempt is made to trace what has happened to the children who presented a negative Wassermann at birth. I have attempted to follow up all the children who were discharged from the service in good condition in order to learn their subsequent history and to obtain a subsequent Wassermann. Unfortunately, this is often extremely difficult, as many of the mothers move away and give their neighbors no information concerning their new location, so that it is impossible to find them, except by accident. We have, however, examined a large number of such children, and among them have thus far found 10 whose Wassermann at birth was negative, but now is positive. In view of these findings, we are forced to the conclusion that a positive Wassermann at birth does not necessarily imply that it will remain so; and conversely, that a negative Wassermann at birth does not necessarily mean that it may not become positive later.

Upon analyzing our material from another point of view, we find upon deducting the children dying within the first month, as well as those who presented signs of clinical syphilis, that we have 10 left, none of whom developed clinical signs of the disease. In five of these the positive Wassermann persisted, while in the other five it had become negative. What will eventually happen to the first group can only be determined by subsequent study.

With these results before us, I feel that we are justified in concluding that the information obtained by the Wassermann made from the fœtal blood at birth is not commensurate with the time consumed, nor the money expended in such investigations. That I am not alone in this opinion is shown by the study of Fildes, who in 1915 reported that he had found a positive Wassermann at the time of birth in 14 out of 1015 children—1.3 per cent. Prolonged observation showed that only one of them subsequently developed clinical syphilis. Seven of the others who were examined later presented a negative Wassermann; while in three other children the original negative Wassermann later became positive. Consequently, he felt justified in drawing conclusions identical with those indicated above.

During the course of years, I had become convinced from the routine microscopic study of the placenta that the syphilitic lesions occurring in it are extremely characteristic, and afford more conclusive evidence of the existence of syphilis than the demonstration of a positive maternal Wassermann, and in general tally fairly closely with the autopsy findings in the child. For this reason I was curious to ascertain in how far the results of the present study would sustain such conclusions.

Chart III gives a graphic picture of the relation between the microscopic findings in the placenta and the clinical and anatomical condition of the child based upon the study of 547 cases. The chart represents the combination of the 449 women presenting a positive Wassermann as shown in Chart I, and the 109 women presenting a negative Wassermann as shown in Chart II, and should include 558 cases. The difference between that figure and the one mentioned is due to the loss of 11 placental preparations. It may here be stated that in the preparation of the material upon which this study is based, the microscopic slides from every placenta concerned were reexamined for the purpose of verifying the diagnosis.

Upon referring to Chart I, we find that a positive Wassermann was originally obtained in 449 women, which later became negative in 172 out of the 178 women who had been efficiently treated, so that 277 cases are available for ascertaining in how far a positive maternal Wasserman corresponds with the existence of the so-called microscopic lesions of syphilis in the placenta, or with the presence of positive clinical symptoms or autopsy findings in the child. Upon analyzing our figures upon this point, we find that the two correspond in 109 instances (B, D, E, G, H, I, J, L, M, N, O, R and S), and fail to correspond in 158 instances (A, C, K, Q and U); while in 10 other instances (F and P), in which the placenta was normal but the dead child did not come to autopsy, no conclusion could be drawn. In other words, in this series the maternal Wasserman tallied with the placental or fœtal findings in 39.35 per cent and failed to tally in 57 per cent of the cases, while in 3.6 per cent the conditions were such that a positive conclusion could not be reached.

It would consequently appear that since there are only four chances out of ten of the results corresponding, it is hazardous to attempt to draw any conclusion concerning the condition of the placenta or of the child from the existence of a positive maternal Wassermann during pregnancy.

Of course it may be objected that such a deduction is not justifiable because of the fact that 102 women who received imperfect treatment during pregnancy had been included in the series, and such an objection must be admitted. On the other hand, information not susceptible to such criticism may be obtained by restricting our inquiry to the 169 women who received no treatment of any kind. Upon deducting the six questionable cases, in subdivision F, we have 163 untreated cases, and of these 67 tallied and 96 failed to tally—42 and 58 per cent, respectively, or essentially the same incidence as before.

The problem may be approached in another way. Upon comparing the placental findings in Chart III with the condition of the children, and taking no account of the Wassermann reaction, it is noted that in 451 instances (A, B, D, J and K), the results tally, while in 56 instances (E, G, H and I), they do not. In addition there are 40 cases in groups C and F, in which the dead children did not come to autopsy, and consequently a positive statement could not be made as to their condition. In other words, in the 547 placentæ concerned, the results tallied in 82.5 per cent, while upon deducting the 40 questionable cases the figure becomes increased to 88.1 per cent.

Furthermore, we may attempt to solve the problem from still another point of view, and we can compare the degree of correspondence according as the placenta was normal or definitely syphilitic. In the 414 cases included in the former category, we note the following results: In 343 the child was discharged alive and well; in 22 it was dead born, but autopsy revealed no evidence of syphilis; while in 22 others autopsy showed that death was due to syphilis, and in 11 the live child presented characteristic lesions of clinical syphilis; while 17 dead children did not come to autopsy. Upon deducting the last 17 cases in which no statement concerning the cause of death is permissible, we find 397 normal placentæ associated with 364 non-syphilitic children. In other words, the results tally in 91.6 per cent. On the other hand, in the 133 cases in which the placenta was definitely syphilitic our figures show 80 dead children with positive autopsies, 7 children with clinical syphilis, 13 children dead but with negative autopsies, 10 children discharged alive and well, and finally 23 children who died but did not come to autopsy. Upon deducting the latter, we have 110 syphilitic placentæ associated with 87 syphilitic children, a correspondence of 79.1 per cent.

From our calculation, it appears permissible to assume that the present investigation indicates that the microscopic examination of the placenta tallies with the clinical and anatomical findings in the child in from 80 to 90 per cent of the cases, which is in marked contrast to the 40 per cent obtained from a positive maternal Wassermann. Consequently, I am glad to find that this study confirms my previous impression, and indicates that the demonstration of the so-called Fränkel's disease in the placenta offers twice as great a probability of giving correct information concerning the condition of the child as a positive Wassermann on the part of the mother, and in the absence of a carefully conducted autopsy constitutes the most reliable means of diagnosis at our disposal.

In 1837 Abraham Colles announced that it was possible for a syphilitic father to engender a syphilitic child by a normal mother, and that the latter would remain immune to infection from her own child, while others might be infected by it. So much clinical evidence was gradually accumulated in support of the correctness of this statement that it became accepted as a dictum and is known as *Colles' law*.

Such an occurrence presupposes infection of the ovum by means of the spermatozoon, with the subsequent development

of immunity on the part of the mother. Presumptive evidence in its favor is afforded by the fact that comparatively few mothers of syphilitic children present a history of primary infection, and frequently give birth to a series of syphilitic children without ever developing signs of the disease. Fournier was an ardent believer in the doctrine, and his advocacy of the occurrence of so-called conceptional syphilis, or *choc en retour*, lent still further support to the probability of paternal infection. Indeed, it may be said that Colles' law was almost universally accepted up to 1903, when its applicability was denied by Matzenauer.

This is not the place, nor will time permit me, to trace the history of the controversy, except to say that with the discovery of the spirochete in 1903 and the description of the Wassermann reaction in 1907, more and more doubt has been cast upon the correctness of Colles' law, with the result that at present most writers, with the exception of Hochsinger, Boas, and Routh, absolutely deny its possibility upon both clinical and so-called scientific grounds.

This reaction was apparently placed upon a firm basis by the contribution of Bab, who pointed out that the relatively large size of the spirochete, as compared with that of the spermatozoon, made it *a priori* improbable that the former could be carried into the ovum by means of the latter. Consequently, he contended that the possibility of paternal infection must be denied, unless the existence of some sporal or intermediate stage of the spirochete were demonstrated. As no such evidence has been adduced, he is inclined to deny the applicability of Colles' law. Furthermore, when the blood of pregnant and puerperal women, who were supposed to exemplify that law, was examined, it was almost uniformly found to present a positive Wassermann reaction. Consequently those interested in the subject concluded that the reason the mother did not become infected by her child was that she was already suffering from the disease, which for some unexplained reason existed in a latent form, and in many instances did not give rise to the usual manifestations.

It must be admitted that such reasoning is very plausible, and to a certain extent justifies the scepticism of those who cry "Away with Colles' law." Indeed, most arguments in its favor are far from convincing, and the recent one advanced by Routh, according to which infection of the ovum is effected by means of "gemmules" derived from the spirochete, is devoid of experimental basis; although, if its correctness could be demonstrated, it would solve many problems.

Notwithstanding the plausibility of the arguments against the correctness of Colles' law, my clinical experience has been such as to cause me to wonder occasionally whether such scepticism is entirely justified; and, while recognizing the difficulties in the way of adducing convincing evidence in its favor, to inquire whether it would not be more rational to regard the question as still *sub judice* rather than as actually disproved.

In the first place, the history of certain mothers of definitely syphilitic children seems to indicate that, if they are really the subjects of latent syphilis, the disease must be of a very different type from that usually encountered. Upon going over the histories of the women presenting a positive Wassermann in our series, only a few could be found who gave any evidence of having had a primary sore, while in less than 20 per cent could a history of the usual secondary or tertiary manifestations be elicited. Furthermore, upon reviewing the patients, who had been in the service for many years, and many of whom had not been treated, we encountered very few who had exhibited the usual sequelæ of the disease.

Menten had a similar experience, and reported that she had been able to elicit only a single history of definite infection in 48 women presenting a positive Wassermann during pregnancy. As the difficulty of detecting the primary lesion in women is generally recognized, it must be admitted that such negative evidence is of less value than in men, but, if a positive Wassermann during pregnancy be accepted as indubitable evidence of the existence of latent syphilis, I cannot rid myself of the impression that the disease must vary markedly from the usual type.

In this connection, one cannot avoid considering the possibility that a positive maternal Wassermann during pregnancy may occasionally be regarded as a manifestation of the establishment of active immunity on the part of the mother against a syphilitic product of conception. Of course this implies the acceptance of spermatic infection, but if such a possibility were once admitted, for which at present there is no scientific basis, it would be easy to comprehend the mechanism by which such immunity might be established from what we know concerning the invasion of the maternal by fœtal tissues and concerning the deportation of chorionic villi during pregnancy.

Suggestive evidence along such lines was afforded by three cases in the present series. In each of them the patients, who presented a positive Wassermann and were not treated during pregnancy, gave birth to syphilitic children, but upon repeating the Wassermann some weeks or months later we were surprised to find that it had become negative. That this is not an isolated experience is shown by the fact that Menten, upon repeating the Wassermann within two weeks after labor upon 26 women, who had exhibited a positive reaction during pregnancy, but who had not been treated, found that it had become negative in 14 and had remained positive in 12 cases. By means of various controls she convinced herself that the conversion could not be regarded as due to errors in technique, and consequently she concluded that it resulted from some deep-seated change in the economy of the women. Furthermore, Dr. Albert Keidel of the syphilitic division of The Johns Hopkins Hospital informs me that such changes are not unusual in his experience. No satisfactory explanation has yet been offered in explanation of this phenomenon, so that all that can be said is that it might be regarded as presumptive evidence in favor of Colles' law, if the possibility of spermatic infection were established.

Turning to another side of the question, it may be asked, if the presence of a positive maternal Wassermann during pregnancy is to be admitted as evidence of the existence of latent syphilis, what can be said of the 65 women included in Chart

II, who presented a negative Wassermann during pregnancy, but yet give birth to 43 definitely syphilitic children? I must confess my inability to answer the question, and, while numerous suppositions might be advanced, I shall content myself with pointing out that as yet we know too little concerning the significance of the Wassermann reaction to feel justified in making dogmatic or *ex cathedra* statements.

In addition to these somewhat dubious reflections, I have seen several patients in whom the theoretical possibility of Colles' law was very great. One of them, who had double ovum twins, appears to afford presumptive evidence in favor of Colles' law associated with superfecundation. The history is as follows: In 1896 and 1897 this colored patient had her fifth and sixth spontaneous labors in our out-patient department. On September 26, 1898 (Out-patient service No. 502), when 27 years of age, she gave birth spontaneously to premature male double ovum twins. The first weighed 4 and the second 4½ pounds; the former was raised, while the latter, which was asphyxiated when born, died in three hours. Autopsy (No. 1153) was done, and the following anatomical diagnosis was made: "Congenital syphilis, syphilitic cirrhosis and interstitial splenitis; interstitial pneumonia; thickening of epiphyseal lines; ecchymoses of epicardium; patent foramen ovale." Microscopic examination of the two placentæ showed that the first was normal, while the second presented typical syphilitic lesions. In other words, one of the double ovum twins was syphilitic, and the other normal.

Upon attempting to find an explanation for this unusual occurrence, the patient was closely questioned, and admitted that she had intercourse both with her husband and with a lover, who was found to be under treatment for syphilis in the genito-urinary service. As all of the six children born prior to the twins had been normal, and as the microscopic examination of the placenta from the sixth was negative, we allowed ourselves to make the somewhat phantastic diagnosis of superfecundation, and assumed that the ovum for the normal twin had been fertilized by the husband and that for the syphilitic one by the lover.

The subsequent history confirmed this supposition, for following the twins the patient had 11 other full-term spontaneous labors with live children, the last one being born on May 8, 1912 (Out-patient service No. 5527), when the patient was 41 years old. Microscopic examination showed that all of the placentæ were normal.

As the twins were born years before the discovery of the spirochete and the Wassermann reaction, no information along such lines was available at that time. In 1916, however, we looked up the patient, and found that she was in good condition and at no time had presented symptoms referable to syphilis. Both she and her husband presented a negative Wassermann, but unfortunately the test could not be made upon the remaining twin as it had died during childhood.

I do not care to be understood as claiming that this case offers irrefutable evidence in favor of Colles' law, but a little reflexion must convince the most sceptical that it is highly suggestive. In this instance, we have records of the microscopic examination of the placentæ from the labor preceding and from the 11 labors following the supposed superfecundation, and all were normal. Furthermore, all of the other children were born in good condition, and were somewhat above the average in weight. Consequently, it may be safely affirmed that the only evidence of syphilis in the remarkable reproductive career of this patient was in one of the double ovum twins. Furthermore, it may be inferred, had the husband been responsible for it, that some of the other children would likewise have presented evidences of the disease, but as this was not the case, it seems permissible to surmise that the lover was responsible for it, in which event it could only be explained as due to paternal infection and that the mother exemplified Colles' law.

In several other instances histories were encountered which can most readily be explained as resulting from spermatic infection and as exemplifying Colles' law.

I shall refer briefly to one such patient (History O. 7279; O. 7766 and O. 8362). In this instance the patient, who previously had five spontaneous labors with normal children, gave birth to a premature macerated syphilitic child in 1916. As her Wassermann was negative, that of the husband was taken and found to be positive. Neither was treated. In 1917 she gave birth to premature macerated syphilitic twins. Again her Wassermann was negative and the husband's positive. Again neither was treated. The following year she returned early in her eighth pregnancy with a negative Wassermann. That of the husband was still positive. The patient was then treated intensively with the result that she had a normal child at full term, with a normal placenta. The husband was not treated. The child was discharged in excellent condition, 112 grams above its birth weight. Unfortunately, however, neither it nor its parents could be located in March, 1920, when we attempted to learn its ultimate fate.

Of course it must be admitted that such a history does not afford conclusive evidence in favor of paternal infection nor of Colles' law, but at the same time it creates a strong presumption in their favor, which to my mind is more convincing than the evidence which is usually relied upon to prove their impossibility.

I shall not attempt to carry the argument further and shall conclude by stating that in my opinion the possibility of spermatic infection and the admissibility of Colles' law have not yet been conclusively proven or disproven, and consequently should be regarded as still *sub judice*.

It is scarcely necessary to add that the collection of the data upon which this paper is based has entailed an enormous amount of routine work on the part of members of the obstetrical staff, as well as of several members of the pathological staff. Consequently, I take this opportunity to express my appreciation of painstaking efforts of a considerable number of collaborators without whose aid the work could not have been completed.

THE CELLS OF THE ARACHNOID

By Lewis H. Weed

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During the past few years there has been evident in the literature a renewed interest in the physiological anatomy of the leptomeninges. The published studies have been primarily devoted to the arachnoid and the pia mater as constituting a membranous pathway for the cerebrospinal fluid; in the earlier years attention was paid merely to the morphology of the cells lining this fluid-containing subarachnoid space. More recently, however, the combined work of several investigators has afforded a more definite and concise conception of the morphology of these lining cells, so that it now becomes possible to present data regarding the changes in form of these cells under certain physiological conditions and to describe other changes resulting apparently from the age-condition of the animal at the time of study. It becomes the purpose of this paper, therefore, to present this conception of the varying morphology of the cells lining the subarachnoid space and to point out as far as possible the conditions which determine these changes in form of the cells.

General Morphology of the Cells Lining the Subarachnoid Space

For a better understanding of the relation of the cells lining the subarachnoid space to the cerebrospinal fluid, a brief anatomical description of the leptomeninges is necessary. Such descriptions have recently been given in other places (Weed [32,34]; Essick [8]); here only the essential characters will be outlined. The outer of the two more delicate investing membranes of the central nervous system—the arachnoidea—is customarily described as a thin continuous membrane, of a glistening external surface beneath the dura, bridging the cerebral sulci and containing beneath it the cerebrospinal fluid. But such a picture of the arachnoid is not complete as it takes account only of the more obvious continuous character of this leptomeninx. In reality this structure should be arbitrarily divided into two parts, the arachnoid membrane (the outer continuous cell-layer investing the whole central nervous system) and the arachnoid trabeculae (the cell-columnns which stretch from the inner surface of the arachnoid to the pia mater).

The arachnoidea becomes, then, a cellular structure with a delicate supporting reticulum, covered on both sides by characteristic cells. From the inner side of this membrane project small delicate trabeculae, upon which are continued the cells forming the inner lining of the arachnoid membrane. The larger of the trabeculæ possess a core of a few connective-tissue fibrils, united on the outer side with the fibrous framework of the arachnoid membrane and on the inner side with the subpial tissues; the smallest trabeculae seem to be composed merely of the cytoplasmic processes of the lining cells. The lining cells, reflected from the inner surface of the arachnoid membrane and continued upon the trabeculæ, become the cells covering the pia mater, for this innermost membrane of the central nervous system is formed by this reflection of the lining cells, supported by a few connective-tissue fibrils and neurogliar elements.

Between these two membranes, the arachnoidea and the pia mater, is the subarachnoid space; it is really merely an intra-leptomeningeal fluid-channel. The passages through this space are interrupted by the arachnoid trabeculæ so that the circulating cerebrospinal fluid must traverse a devious, tortuous, but continuous channel. Essick [8] (p. 380) has likened the pia mater and arachnoid to "a living sponge accurately filling the irregularities between the brain and the dura. . . . . Just as the spaces in a sponge are in free communication, so the cerebrospinal fluid is normally continuous everywhere."

The occurrence of this web-like network in the subarachnoid space represents a partial persistence of certain characteristics of the embryonic structure of the leptomeninges. Studies on the development of the cerebrospinal spaces have indicated that the meningeal channels arise by a dilatation of the meshes in the perimedullary mesenchyme, resulting in rupture of many of the cytoplasmic strands and in the formation of the permanent arachnoid trabeculæ (Weed [33]). The cells of this mesodermal tissue become modified in their development to form the typical lining cells of the subarachnoid space. Likewise, similar mesodermal elements differentiate to form the cells lining the inner surface of the dura and the outer surface of the arachnoid membrane. With such a developmental history it seems quite proper to term them "mesothelial" cells.

Morphologically, these covering cells of the leptomeninges are of a low flat form, with large, pale, oval nuclei. The chromatin net in the nucleus is usually only indistinctly made out. With ordinary histological stains the cell boundaries are not visible, but definite intercellular lines may be demonstrated by silver nitrate (Key and Retzius,[7] Essick [8]). The cytoplasm of these flattened mesothelial cells contains fine granules which are of larger size near the nucleus as shown in the excellent plates of Key and Retzius. These authors also described in the arachnoid cells fat-like material, which, though staining with osmic acid, did not give the dense black of the ordinary fat-droplet.

Study of the morphology of the cells of the arachnoid in ordinary histological sections is by no means as satisfactory as when made upon spread-preparations. In these, the arachnoid membrane is dissected away under a binocular microscope by sectioning the arachnoid trabeculae as near to their pial attachment as possible. The delicate membrane is then mounted on a slide with the outer surface down, so that the isolated and freed arachnoid trabeculæ are presented for study. This permits direct observation of the cells of the inner surface of the arachnoid and particularly of those covering the arach-

noid trabeculæ. The dissection of the arachnoid in the spinal region is not difficult but over the cerebral hemispheres the procedure is complicated by the delicacy and fineness of the arachnoid mesh. With this method excellent pictures of the typical flat mesothelial cells can be obtained.

These mesothelial cells are usually so placed on the surface of the leptomeninges that they are of only one cell in thickness. At times the number of cells in a given area of membrane is increased and the cells are of more than one layer in thickness; these areas of reduplication of the cell-covering become evident in both histological sections and in spread-preparations. Under normal conditions the cells of the leptomeninges are of the low, flattened type indicated, with a granular cytoplasm and pale oval nuclei. Everywhere throughout the cranial and spinal regions this typical morphology holds.

Changes in the Cells under Physiological Activity

It was noted in 1917 that the general morphology of the cells of the leptomeninges "depends apparently not only on their situation but also on their physiological state." (Weed,[14] p. 467.) The evidence for the latter part of this statement was based upon the changes in the arachnoid cells in response to the injection of particulate matter into the subarachnoid space. Within a few hours after such injections of carbon granules (India ink) the cells of the arachnoid were found to contain the particles; with cinnabar a similar cellular reaction was observed, phagocytosis of the granules in this case being not as widespread nor as active as with the wholly inert carbon granules. Under such experimental conditions, with the cerebrospinal fluid filled with the foreign particulate matter, the cells became larger, rounded up and assumed somewhat cuboidal forms. Largely because of the frequency with which these appearances of cuboidal form were met with in the specimens injected with particulate matter, the general descriptive appellation of "low cuboidal cells" was applied to the cells of the leptomeninges. Although, when used, this term was qualified by reference to the customary flat morphology, it does not, in view of the more extensive knowledge of the physiological reactions of the cells now possessed, seem longer justified. Additional argument for the use of another adjective in the descriptions of the cells of the arachnoidea has been based on the changing morphology of these cells as they invade the dura mater in certain restricted areas and become the typical cell-columns of the pachymeninx (Schmidt,[10] Weed[15]). Such cell-columns are normal structures; their occurrence makes the qualification that the morphology of the cells of the arachnoid depends not only on their location but also on their physiological state justifiable. Yet in the description of the cells lining the subarachnoid space, emphasis on the normal flat character of the resting or inactive cell should continue to be placed.

Following these primary observations that the cells of the arachnoid changed their morphology in response to particulate matter in the subarachnoid space, Essick[3] greatly extended our knowledge of the physiological response of these cells to other kinds of particulate matter. His observations were made almost entirely on spread-preparations of the spinal arachnoid; the isolated trabeculae afforded an excellent opportunity for the study of the reactions of the isolated cells. Staining of the isolated portion of the arachnoid membrane with dilute solutions of toluidin blue permitted satisfactory histological studies.

The most striking reactions of the arachnoid cells Essick found to follow the subarachnoid injection of laked blood. The blood was prepared by laking with distilled water and restoring the solution to isotonicity by adding suitable quantities of a stronger solution containing sodium chloride, potassium chloride and calcium chloride. Such subarachnoid injections of partially hemolyzed red corpuscles caused a marked sterile meningitis within six hours; this inflammatory process subsided rapidly, becoming almost quiescent at the end of 48 hours. During the latter part of this reaction, the cell-content of the cerebrospinal fluid changed from a polymorphonuclear to a large mononuclear element. These large mononuclear cells possessed all of the characteristics of the amœboid macrophage, to use Metchnikoff's[8] designation as qualified by Evans.[4]

Under these experimental conditions Essick found great changes in the morphology of the cells of the arachnoid, when studied in spread-preparations. The protoplasm of the cells first increased in thickness so that the nuclei no longer stood out sharply on profile view, and this increase in volume of protoplasm about the nucleus continued, while the nuclei became more circular in outline. Gradually the attachment of these cells to the framework of the trabeculae or membrane became less broad and inclusions of fragmented red cells appeared within the cytoplasm. Many of these phagocytic cells then became detached, to form the free round phagocytes, identified in the cerebrospinal fluid. Similar changes in the morphology of the covering cells were made out in the outer surface of the arachnoid membrane, following the accumulation of blood in the subdural space. When the phenomenon of budding-off of these cells was outspoken, many mitotic figures were seen in the membrane.

Essick further studied the reaction of the cells of the arachnoid to dilute suspensions of carbon and cinnabar granules. While the formation of free macrophages was not as frequent as with the laked red cells, the same process of phagocytosis of granules and swelling of the protoplasm was observed. Although the reaction of the cells of the arachnoidea to these granules was not as vigorous as with the blood, the same biological change in morphology to both varieties of particulate matter seemed established. Schott[11] and later Goldman[5] had shown that both the pleural and the peritoneal surfaces could furnish free-moving phagocytic cells; the same physiological reaction, as with the mesothelial cells of true serous cavities, occurred in the mesothelial cells of the subarachnoid space.

In studies of the reactions in the subarachnoid space to infective processes, Ayer,[1] working contemporaneously with Essick, observed at times an essentially similar change in the morphology of the lining mesothelium. In many early acute cases of experimental meningitis the cellular exudation in the meninges was of the large mononuclear type; Ayer stated,

from a study of transitional stages in the cells still attached to the membrane, that at least some of these cells represented macrophages derived from the arachnoidea. In the presence of the toxins of such early infections, the mesothelial cells became larger, with accumulation of the cytoplasm about the nuclei; the cell-attachments became less extensive and in sections the cells appeared as if ready to be detached. The many complicating factors in such infections, however, rendered more exact statement of the reactions of the cells of the arachnoid impossible.

The evidence, then, supports the view previously expressed (1917) that the morphology of the mesothelial cells of the arachnoid depends at least in part upon the physiological state of the cells. While normally the cells are of a flattened, very low type everywhere over the arachnoid membrane, they become, in response to the stimulus of particulate matter (carbon, cinnabar, fragmented red cells, etc.), as well as in certain acute infective processes, enlarged and elevated, with inclusions of foreign material within them.

Changes in Arachnoid Cells Conditioned by Age

In 1859, Meyer[1] described abnormal aggregations of cells in the arachnoid as "Die Epithelsgranulationen der Arachnoidea," a post-mortem finding in the brains of patients suffering from mental disease. These observations of Meyer's have not been extensively quoted in the literature and the phenomenon has not been satisfactorily described. In a paper by Cushing and Weed[2] "a peculiar proliferation of the arachnoid mesothelium of a cat" was recorded. This cellular overgrowth was observed in but a small proportion of laboratory specimens (man and ordinary laboratory mammals); no ages were given for the animals in which the process was noted, though the first animal was recorded as a "young adult" cat. It was thought at the time that the proliferative "process was almost invariably associated with the deposition of lime salts in the membrane." Subsequently, Essick[3] described similar areas of thickening in the arachnoid membrane: "Distributed throughout the brain and cord are clusters of closely placed nuclei within the arachnoid membrane. . . . . Such areas are irregular in shape, size and distribution. . . . . They represent normal structures and, like the arachnoid trabeculæ, become the seat of calcium deposits with the advancing age of the animal. In no sense should they be mistaken for a cellular proliferation in response to a degenerative process."

It has been my intention for several years to study these cell-masses in the arachnoid membrane more thoroughly and to present as far as possible a more complete description. As isolated fragments of evidence accumulated in the study of the leptomeninges both in normal and in infected animals, it became more apparent that the age of the particular animal was one of the most important, if not the determining factor in their occurrence. The cell-nests had been met with in practically all of the laboratory animals as well as in man; their occurrence in all of these species indicated that their biological significance was great.

Recently a series of cats was chosen with particular reference to the age of each animal as determined by general appearance, condition of teeth, etc. While this determination of age of the cats could never be made with thorough exactness, the animals could roughly be classed according to their age without much error. Each cat, as soon as the determination of approximate age was made, was weighed and then killed with ether. The thorax was opened immediately thereafter and a cannula inserted through the left ventricle into the aorta. Customarily the blood was washed out by perfusion with warm Ringer's solution; on the venous side an outlet was provided by making a small incision into the tip of the right atrium. As soon as the perfusing fluid became clear, an injection of 10 per cent formalin was made through the aortic cannula and the atrial opening clamped. About one liter of fluid was usually injected under a pressure of 900 millimeters of fluid. Subsequently, the calvarium and arches of the vertebræ were removed and the central nervous system was further fixed by immersion in formalin. Finally, the bony structures were entirely removed, and the central nervous system, still surrounded by intact meninges, was left in the formol until studied. When examined, the dura over the cerebral hemispheres was carefully opened and the underlying arachnoid lightly stained with a dilute solution of toluidin blue or of methylene blue. The dura of the spinal cord was likewise carefully opened and the same stain applied to the exposed arachnoid membrane.

When examined under the binocular microscope, the meninges of this series of cats could be easily divided into two groups: those which showed cell-accumulations in the arachnoid membrane and those which presented a smooth, regularly staining surface. In the former group, the arachnoid membrane, particularly over the hemispheres where the study could be made with great ease, showed irregular, discrete, more densely stained areas. Even in the gross, these areas of deeper stain could be made out, and the exposed membrane over the cortex presented a mottled appearance. There was usually great variation in the shape and size of these more densely stained areas: at times they were circular with sharply defined borders; again they were irregular and faded gradually off into the adjacent thin arachnoid membrane and occasionally they represented mere fusiform thickenings of the membrane. In some animals, only a few, small, ill-defined masses appeared over the whole arachnoidea; in others the membrane was thickly covered with the more densely stained nodules. Frequently in the more definitive areas of cell-accumulation oval or rounded glistening bodies were identified in the midst of the deeply stained masses; structurally these glistening bodies seemed unlike the remainder of the membrane.

The distribution of these more densely stained areas in the arachnoid membrane was fairly well demonstrated by such macroscopic examinations. In those animals in which the masses were few in number and quite minute, the greater number of nodules, in the cerebral portion of the nervous system, appeared in the arachnoid as it bridged the larger sulci, particularly in the cruciate and sylvian regions. In the spinal cord, the distribution seemed more regular and without great

differences in localization; in general the ventral surface of the cord seemed practically uninvolved in the process, while the dorsal half showed many of the deeply stained areas. Quite similarly in the brain, the ventral surface of the brain stem was practically always free from the nodules, even though the cerebral cortex might be extensively covered; in only one case of the series was a typical more densely stained area made out on the basilar surface.

These more densely stained areas, so clearly shown in this relatively gross examination, were easily resolved, under higher powers of the binocular microscope, into deeply stained nuclei, rather closely packed together. Elsewhere than in these areas of differentiation, the arachnoid membrane presented more or less regularly placed nuclei, not closely approximated to one another but separated by appreciable distances. In the densely stained area, the nuclei were closely gathered together and separated by but little lightly stained cytoplasm. Occasionally in the midst of such a nuclear aggregation, there could be made out a diffusely stained, more or less homogeneous substance without definite structure. And again, oval or spherical masses taking a fairly dense stain stood out in contrast to the adjacent closely approximated nuclei.

When blocks for histological sections were cut so that the leptomeninges remained attached to the underlying brain substance, the characteristics of these more densely staining masses were brought out even more vividly than by examination under the binocular microscope. Sections both perpendicular and tangential to the nervous tissue were employed for this purpose; in general, the perpendicular sections afforded the better opportunities for study. In such preparations, the more densely stained areas seen in the gross examination were easily identified as localized thickenings of the arachnoid membrane, characterized by considerable aggregations of closely packed nuclei and varying amounts of protoplasm (Figs. 1, 2, 3 and 4). These nodular masses varied markedly in size and in form, being at times sharply delimited from the adjacent membrane or diffusely merging with it. The nuclei were in general somewhat more rounded than the typical oval nuclei of the arachnoid cells elsewhere; the chromatin-content in some of the nuclei was diminished, while in many other cells there seemed a slightly increased amount. The cytoplasm of the cells comprising the nodular swelling contained some granular inclusions near the nuclei, but not in as definite form nor so regularly distributed as in the ordinary arachnoid cell (*cf.* Key and Retzius[7]). In the cell-mass, intercellular borders were not distinguishable by the ordinary histological stains. In general the nuclei were very closely packed together (Fig. 1) but in other areas the relative amount of cytoplasm was greater (Fig. 4). Even in the nodular swellings themselves the tendency to irregular massing of the nuclei was great (Figs. 2 and 3) and formations of small whorls were evident.

Many of these arachnoid cell-clusters appeared in section to be very sharply circumscribed aggregations of cells; the transition was not wholly abrupt but there occurred a small prismatic area of enlargement at each pole of the oval swelling. The occurrence of such a cell-mass did not seem to affect the cells in the immediate neighborhood; these showed no histological evidence of change from their customary flattened form. In other situations, even at times in the same animal, the typical arachnoid cell-cluster formed merely the most prominent part of a diffuse thickening of the whole membrane as illustrated in Fig. 2 and to a lesser extent in Fig. 3. Certainly in these cases, the tendency toward change was met by the body in a slightly different way, so that the cell-alteration in the one case was confined to a very definite locus and in the other to a more diffuse and extensive area. No difference in significance of the two types of swellings has been made out.

Certain phases of the relations of these arachnoid cell-clusters were of particular interest. In the case of the localized, definitive enlargement the general impression derived from examination of the sections was that the whole cell-cluster could be easily shelled out from the membrane (Fig. 1). With the more diffuse swelling (Fig. 3) this impression was not given, the diffuse cell-cluster remaining an integral part of the membrane in every way. With both types of swelling, however, the inner surface blended intimately with the delicate and irregular arachnoid trabeculæ (Figs. 1, 2, 3 and 4). But most interesting of the relations of these nodular masses were those of the outer surface of the membrane. It has been known for some time (*cf.* Schmidt,[10] and Weed[13, 14]) that there exist, scattered irregularly throughout the subdural space, prolongations of the arachnoid membrane into the dura; these cellular extensions are connected for the greater part with arachnoid granulations in the dura. In other places, the arachnoid seems to be incompletely separated from the dura so that small but definite areas of fusion exist—a phenomenon which might well be predicted by consideration of the embryology of the two membranes (Weed[15, 16]). The frequency of attachment of the arachnoidea to the dura becomes apparently much greater in the sites of development of the typical cell-clusters. In many instances in the present series of animals it was almost impossible to separate the two membranes in such localities without damage or lifting away of the cell-cluster; Fig. 4 shows well the result of such an artificial separation. Here the outer surface of the arachnoid membrane is shown above; the cell-nodule has been lifted away from its bed in the arachnoid membrane and the loose outermost strands of the surface have been torn away. The connection of such cell-clusters to the dura did not seem to be very firm but the adhesion was definite and important.

If the arachnoid cell-cluster be looked upon as an area of active hyperplasia of the membrane, one should expect to find many mitotic figures in the cells of the mass. Extended search for such evidences of cell-division has been made, but only very rarely could such a figure be identified. It must be assumed therefore that the initial processes resulting in the formation of such cell-clusters were probably slow and continued over a relatively long period of time; the cell-growth is in no sense to be considered a rapid, wholly unrestrained proliferation of the covering cells of the membrane.

When the occurrence of these arachnoid cell-clusters was related to the records of the approximate ages of the cats in the series, it became at once apparent that the very old animals in every case showed such cell-aggregations in the arachnoidea. In the very young animals, the cell-clusters were never found; in adults, in which senile changes were not obvious in the general examination, the cell-clusters were occasionally encountered. It seemed obvious, therefore, that the presence of the cell-clusters was conditioned upon the age of the particular animal; their absence in the immature and invariable appearance in the old indicated that during adult life there occurred changes which finally resulted in the development of the cell-clusters. In the adult animal, the tendency to the cell-hyperplasia in the arachnoid increased as the animal grew older. Variations in the number of the cell-clusters were found in animals equally senile; no significance could be attached to this finding. The relation of the age of the animal to the occurrence of such cell-clusters seemed established.

Ultimate Changes in the Arachnoid Cell-Clusters

It becomes, of course, a matter of interest to determine the fate of these relatively frequent cell-clusters in the arachnoid membrane. Meyer[9] described, in the epithelial-granulations he recorded, the occasional infiltration with calcium salts. Cushing and Weed[2] noted that the cell-clusters were a common site of the process of calcification in the arachnoid. They stated (p. 368) that "though the proportion of laboratory specimens in which we observed examples of cellular hyperplasia was small, the process was almost invariably associated with the deposition of lime salts in the membrane." Essick[3] presented a similar view when he wrote (p. 381) that the arachnoid cell-clusters "represent normal structures and, like the arachnoid trabeculæ, become the seat of calcium deposits with the advancing age of the animal." Study of the present series of specimens has led to the view that calcification represents but one phase of the ultimate fate of these cell-nodules; a second somewhat more striking reaction is represented, not on the side of degeneration (as in calcification) but in an outspoken proliferation which apparently results in neoplasms.

Cushing and Weed[2] gave a detailed account of the formation of calcareous nodules, tracing the histological history of the psammoma bodies or corpora amylacea. The early evidences of the process of calcification were shown to occur at times in the nuclei of the arachnoid cells; at other times, in the cytoplasm of the cells. Notation was made of the formation of whorls in the areas of hyperplasia of the arachnoid mesothelium; this arrangement of the cell nuclei was especially frequent about the calcareous nodules.

With this view, expressed five years ago, the deductions from the present study largely coincide. But the initial formation of the arachnoidal cell-cluster in the membrane may be best considered as a phenomenon of advancing age in the individual; such cellular nodules represent an almost invariable process of hyperplasia in senescence. Apparently the cell-nests reach only a limited size; they never become, as judged from the many specimens studied, very large, usually attaining a maximum diameter of one to three millimeters. Many seem to persist without showing any signs of degeneration, remaining of a constant size or possibly slowly increasing in one or other diameter. Others in the same animal undergo degeneration with the infiltration of calcareous material. In no specimen studied, however, did more than three-fourths of the cell-clusters show signs of this calcareous degeneration; it is highly probable, however, that any extreme case might exhibit calcification in practically all of the nodules.

The degeneration of the cells of these nodules in the arachnoid may be connected with the relatively poor vascular supply to the arachnoid membrane. According to the best anatomical observations the arachnoidea is practically devoid of capillaries and in these cell-clusters there seems to be no evidence of new formation of a vascular bed. The earliest form of degeneration consists apparently in a change of the substance of the cells into an amorphous hyaline-like material in which only vague shadows of cell-bodies exist (Fig. 5). Whether this is a stage in the formation of a real calcified body cannot be told; certainly, as shown by Cushing and Weed, the calcareous deposition may occur in nucleus and in cytoplasm in very minute but histologically detectable masses. But these areas of amorphous degeneration many times occur in the same arachnoid cell-cluster as a definite calcareous body (Fig. 6); the two processes may then proceed simultaneously, the one possibly representing the initial stage of the other.

When the process of calcification in the cell-clusters becomes outspoken, the calcified body may assume the shape of a rather imperfect sphere in which on section concentric rings are evident (Fig. 7). In other nodules the calcium deposit is very irregularly placed (Figs. 6 and 7), the deposition being incomplete or involving only a portion of the area of degeneration. At other times the area of calcification in such an arachnoid cell-cluster may be quite limited and the remainder of the cell-cluster relatively unaffected. Such a condition is illustrated in Fig. 8, in which a large calcareous body appears to the left, while elsewhere in the cell-cluster the nuclei show but little evidence of change, though the more darkly shaded areas seem to be the seat of an early change. Surrounding the definite calcareous masses, the arachnoidal cells frequently show typical whorl-formation as in Fig. 7. In other cases, however, the area of calcification may involve the whole cellular area, the cells not being grouped into whorls. While this tendency toward whorl-formation is so frequently met with about the calcified bodies, it must not be interpreted solely as a reaction to the calcified body but rather as an expression of a peculiarity of the cell-growth in the arachnoidea as well as elsewhere. For the occurrence of similar whorls in the cell-clusters of the arachnoid (Figs. 1 and 3) has already been emphasized.

That calcareous degeneration is not the sole pathological change to which these arachnoidal cell-clusters are subjected was demonstrated by the findings in two animals in this series of cats. In both of these, masses involving the cerebral arachnoid were found on opening the dura; in the first animal the initial impression was that the arachnoidal cell-clusters were

exceptionally large and exceptionally well differentiated from the membranous expansion of the arachnoid, while in the second obvious masses of new-growth were apparent. In this second animal two relatively large tumors of rather tough, glistening tissue were found, the one in the left sylvian fissure while the other was closely attached to the falx cerebri on the same side, displacing the substance of the cerebral cortex and blending intimately with the leptomeninges. In the first of the two cases, the diagnosis from gross examination was impossible; in the second cat, a tentative diagnosis of cerebral neoplasm seemed justified.

Microscopically, the leptomeninges in the first of these two animals showed a rather small, definitely circumscribed new-growth with all the characteristics of the so-called dural endothelioma in man. The mass showed an increased number of rather deeply stained, oval nuclei with relatively sparse cytoplasm; the cells were for the greater part arranged in whorls and surrounded in many places typical calcareous bodies (Fig. 9). The tumor seemed quite well vascularized, and in the fixed tissue spaces between the whorls are numerous. The mass was situated in the outer portion of the arachnoid membrane, leaving a well-marked subarachnoid space between it and the brain-substance. The dura covering the mass showed a slightly roughened area of attachment without evidence of invasion of the dura by an arachnoid cell-column; the line of cleavage, however, on lifting the dura was between the dura and the tumor. This phenomenon of partial attachment of the abnormal cells of the arachnoid to the dura was essentially similar to the attachment of a typical cell-cluster to this membrane. Histological examination of other blocks taken from the brain of this animal showed many arachnoidal cell-clusters, some of which contained calcareous bodies, but no other tumor mass.

Similar, but much larger, endotheliomata were found in the second of the two cats. Here in both of the areas in which macroscopically abnormal growths were made out, tumors of the same type, rather widely separated and in no way connected with each other, displaced the brain substance. The tumors seemed on microscopic examination to be somewhat more actively growing than in the case of the small tumor in the first cat (Figs. 10, 11 and 12). The tumor cells were for the most part arranged in whorls of greater or less size; occasionally (Fig. 12) a large whorl contained within it a series of these smaller concentrically arranged cell-nests. Histologically, the individual tumor cells were of typical form with oval, rather deeply stained nuclei and with varying amounts of cytoplasm. The vascular channels, comprised of rather large vessels with thin walls, were quite abundant in these two larger growths. Small round or irregular calcareous masses were very common (Figs. 10 and 11); about these bodies the cells were arranged in the characteristic whorl. In other places the calcium was laid down in a more or less extensive plaque; in Fig. 12 a large crescentic mass covering a half of the periphery of the spherical tumor is shown. An extensive connective-tissue framework of the tumor was revealed by Mallory's stain which outlined the cell-whorls with circular fibers. In every way, the histological picture was that of the typical "dural endothelioma" of man.

The relation of these tumors to the dura mater are of interest. In the first of the tumors (Fig. 9), the separation on raising the dura came between dura and arachnoid; there existed, however, a rather intimate but easily broken attachment of the tumor to the dura. This adhesion was easily made out in microscopic sections; some evidence of the torn filaments appears in the photomicrograph. In every way, the dural relations of this new growth were quite similar to those shown in Fig. 4, where the separation of the dura and arachnoid resulted in the lifting up of the arachnoid cell-nest from its bed in the membrane. The connections of the endotheliomata to the dura in the second cat were quite different from those in the first. Here (Figs. 10 and 11) the new-growth was very closely attached to the dura, so that the tumor appeared to have arisen from this dense fibrous membrane. The connective tissue of the endothelioma blended, as shown by Mallory's stain, with the dural connective tissue.

This intimate attachment of the tumor to the dura is quite similar to the usual arrangement in man; it has resulted in the designation of these tumors as "dural endotheliomata." Consideration of the findings in the case of the arachnoidal cell-nests and of the endothelioma in the first of the two cats leads to the suggestion that, in at least some of these instances, the dura attachment is secondary to the initial growth of the tumor, dependent upon other factors, such as the possible subsequent development of vascular channels from the dura mater.

Of course it is very difficult to decide with exactness the point of origin of any differentiated tumor mass. In the growth of the endotheliomata of the meninges, the evidence at hand suggests strongly that they arise as a proliferative phenomenon from the initially slowly growing arachnoid cell-cluster. The somewhat similar type of cell, the occurrence in both growths of typical cell-whorls, their common calcareous degenerations, and their apparent identical topographical relations in the arachnoid membrane seem greatly in support of this conception. In a somewhat different sense, Schmidt[10] considered the endotheliomata to be arachnoidal in origin and an essentially similar viewpoint was expressed by Cushing and Weed[2] as follows (p. 371): "The so-called dural endotheliomas show histologically the same cellular arrangements with calcareous and osseous depositions that are commonly found in the arachnoid and therefore take their origin in all probability from the mesothelium of this membrane." To these hypotheses of arachnoidal origin of the endothelioma, the present findings in the cat add support; the greater evidence to-day seems to depend upon the very common occurrence of the arachnoid cell-clusters in adult and old animals. Between the true tumor and the cell-cluster, are transitional types—large elongated cell-clusters in the arachnoid in which the cells resemble more the tumor cell, of apparently more active growth. Such specimens were found in two of this series of cats; while classed with the arachnoid

cell-clusters, their appearance suggested strongly the endothelioma.

The arachnoidal origin of such endotheliomata may at times be really intra-dural, from the columns of arachnoid cells normally present in the pachymeninx. These intra-dural collections of the arachnoidal mesothelial cells are connected with the prolongations of the arachnoid membrane into the dura; the cells are rather closely packed together and surrounded by the dense fibrous tissue of the dura. Histologically these cell-columns are essentially similar to the arachnoid cells in a typical cell-cluster. Schmidt's [10] studies led to the conclusion that the endotheliomata were arachnoidal in origin, and were particularly related to the intradural arachnoid cells covering the Pacchionian granulations or those invading the dura as arachnoid cell-columns. Wojna [17] has pointed out the relation of similar aggregations of cells to physiological herniation of the brain; these observations are of interest in showing the importance of such intradural aggregations of cells. If the endothelioma should arise from such a cell-nest within the dura, its connection to the dura would be intimate and primary; if from the cell-cluster in the membrane it would be possibly equally intimate but in every respect secondary.

Discussion and Summary

From the data gathered together in the foregoing pages it becomes possible to present a more definite conception of the cells of the arachnoidea. While normally in the resting state, the cells covering the arachnoid membrane on its outer surface and lining the subarachnoid space are of a low flattened form, this morphological character changes with alteration of the physiological state and in the altered conditions of advancing age. Thus in response to the stimulus of particulate matter, the arachnoidal cells enlarge, become phagocytic, and multiply, some becoming under appropriate conditions free-moving macrophages in the subarachnoid space. An essentially similar response of the cells occurs in the early stages of certain acute infections of the meninges. That the cells of the arachnoid react in a similar way to such widely varying stimuli would indicate that the phenomenon is the morphological expression of an essentially similar alteration in the functional state of the cells.

A second change of significance in the cells of the arachnoid membrane is that of the slow hyperplasia of the mesothelial elements to form well localized cell-clusters in the membrane. These cell-masses occur in adult animals, particularly in those in which signs of advanced age are apparent. The phenomenon of this cell-overgrowth has not been observed in young animals in any instance; the hyperplasia is determined apparently by the age-condition of the particular animal. These cell-clusters are the seat of the very common process of calcification in the arachnoid membrane, and the cell-cluster should be considered to be the primary change in the membrane, permitting subsequent degeneration and infiltration with calcium. On the other hand, in certain of the series of cats described, a much more intensive proliferative process had taken place in the meninges, to result in the formation of typical endotheliomata. The evidence indicates that certainly, in at least some of the cases, these new-growths represent similar but much more hyperplastic reactions of the same arachnoid cells than are involved in the formation of the much smaller, more slowly growing arachnoid cell-cluster. The exact cell-origin of such new-growths could not of course be told with exactness, but many features of similarity in the two types of growth suggest that this true new-growth is the proliferative end-result of the arachnoid cell-cluster while the very frequent process of calcification in these cellular nodules represents the degenerative.

The significance of the very slowly growing arachnoid cell-clusters must remain somewhat speculative. Their invariable occurrence in the older cats suggests that this cell-overgrowth resulting in the formation of such cell-clusters is merely a phenomenon of senescence. The cell-clusters are found quite frequently in adult animals, in which the evidences of old age are not present; they have not been found in young animals. This age-condition seems quite similar to that described by Goodpasture and Wislocki [6] in their paper "Old Age in Relation to Cell-Overgrowth and Cancer." In their series of 15 senile dogs, tumors were found in all of the animals, usually in more than one place (most frequently in liver, spleen and adrenals). The uniform and coincident occurrence of these tumors, and a certain similarity in the mode of formation and growth, indicated a common causative agent. If senescence be assumed to be this common causative agent, the cell-clusters of the arachnoid may well be classed in the same category.

The almost invariable occurrence of the arachnoid cell-clusters in animals of advanced age makes one hesitate to class them as abnormal structures but rather consider them as normal structures developed in senescence. In favor of classifying the structures as pathological are the evident hyperplasia of the normally flattened arachnoid cells, the tendency of the cell-cluster to show signs of calcification and the suggestive relationship of these nodules to endotheliomata. On the other hand, the inevitable occurrence of these cell-clusters in old animals leads one to the idea that, if not a normal change, the slowly progressing overgrowth must be viewed as a constant change in senescence. Calcification in the choroid plexus and epiphysis cerebri has been considered to be so frequent as to be practically a normal phenomenon of advancing age by Virchow;[18] certainly in such a degenerative process cell-changes must have preceded the actual degeneration. It seems most fair to look upon the arachnoid cell-cluster as an overgrowth of the arachnoid mesothelium, conditioned by the age of the animal and representing an almost inevitable alteration of advancing age.

Conclusions

The arachnoid mesothelial cells are normally of a low flat type but their morphology depends upon the particular physiological state of the cells at the time of examination. Under the stimulus of particulate matter and in acute infections, the cells increase in size, become phagocytic and at times form free-moving macrophages. Other changes in the growth of the

arachnoid cells lead to the almost invariable formation, in the older animals, of cell-clusters,—slowly progressive overgrowths, at times undergoing calcification and less frequently seemingly related to the formation of endotheliomata. Hence, the morphology of the cells of the arachnoid may be said to depend not only upon the location of cells (as on the membrane or in an intradural cell-column) and upon the physiological state of the cells (as under the stimulus of particulate matter and infections), but upon the age-condition of the animal (as in the arachnoid cell-cluster).

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DESCRIPTION OF FIGURES

Fig. 1. Photomicrograph of section of arachnoid membrane covering posterior portion of left lateral sulcus of brain of old cat, showing thickening of mesothelial cells to form arachnoid cell-cluster. Above the membrane is the subdural space; below, the arachnoid trabeculæ appear traversing the subarachnoid space. (×210.)

Fig. 2. Photomicrograph of section similar to that of Fig. 1, but taken from anterior portion of lateral sulcus of same animal. A similar arrangement of arachnoid membrane with cell-cluster is given. (×120.)

Fig. 3. Photomicrograph of section of arachnoid membrane covering left sylvian fissure, from same cat as those of Figs. 1 and 2. A typical arachnoid cell-cluster is illustrated; on the right the demarcation from the thin membrane is sharp, while on the left the swelling is continued somewhat diffusely into the membrane. (×120.)

Fig. 4. Photomicrograph of section of arachnoid membrane (over right parasylvian fissure) from brain of rather old but vigorous adult cat. Above is the dural surface of the membrane; the characteristic cell-cluster is shown lifted out in part from its depression in the membrane. Mesothelial strands attaching arachnoid to dura mater lie free in the subdural space. (×190.)

Fig. 5. Photomicrograph of section of leptomeninges and brain (posterior portion of left parasylvian gyrus) of an obviously old cat. In the midst of a typical arachnoid cell-cluster is an area of more or less homogeneous material; this area of degeneration shows no evidence of infiltration with calcium. (×120.)

Fig. 6. Photomicrograph of section of arachnoid membrane over left lateral sulcus, from brain of same animal pictured in Fig. 5. The right half of the arachnoid cell-cluster here shown has undergone degeneration—in part of a homogeneous character—but elsewhere a beginning infiltration with calcium is evident. (×120.)

Fig. 7. Photomicrograph of section of arachnoid membrane and brain (right parasylvian gyrus) from an obviously old cat. The arachnoidal cell-cluster exhibits, in addition to several small and irregular calcareous deposits, typical spherical calcified bodies. Around certain of the calcareous nodules whorl-formation in the mesothelial cells is indicated. (×55.)

Fig. 8. Photomicrograph of section of arachnoid membrane (anterior portion of left anterior sulcus) of brain of same animal as in Fig. 7. A large calcified body is shown on the left side of the arachnoid cell-cluster with no evidence of cell-reaction around it; in the remainder of the cell-cluster areas of beginning calcareous infiltration may be made out. (× 120.)

Fig. 9. Photomicrograph of section of leptomeninges and brain (coronal sulcus) of obviously old and weakened cat. Above in the leptomeninges is shown an early, well localized endothelioma with typical cell-whorls and calcified bodies. (×38.)

Fig. 10. Photomicrograph of section of dura and tumor-mass from brain (left sylvian fissure) of obviously old cat. The tumor is a typical endothelioma and is intimately attached to the dura, which appears above. (×40.)

Fig. 11. Photomicrograph of section of dura and tumor from brain (displacing right marginal gyrus) of same animal as that from which Fig. 10 is taken. Above is shown the dura of the falx cerebri, with the diverticula of the superior sagittal sinus. The typical "dural endothelioma" with its cell-whorls and calcareous bodies is intimately connected with the dura of the falx. (×52.)

Fig. 12. Photomicrograph of section of tumor mass taken from brain in same region as that given in Fig. 11. A large cellular whorl, capped on the left by a crescent of calcareous material, is shown in the center of the photomicrograph; localized cell-whorls occur in the midst of the large whorl. (×40.)

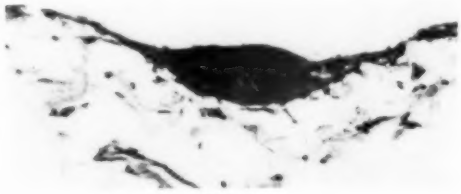
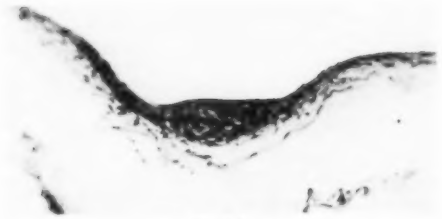


FIG. 1.



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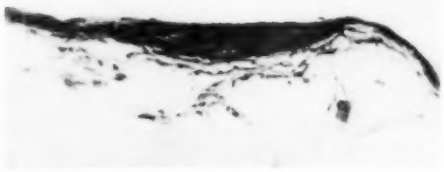

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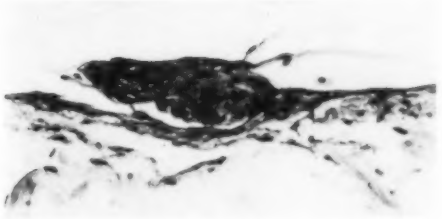

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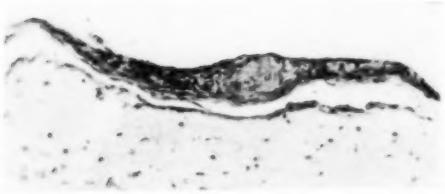

FIG. 5.

FIG. 6.

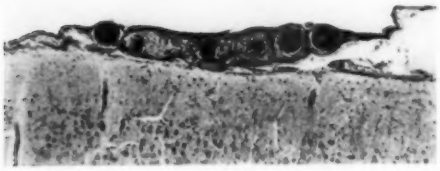

FIG. 7.

FIG. 8.

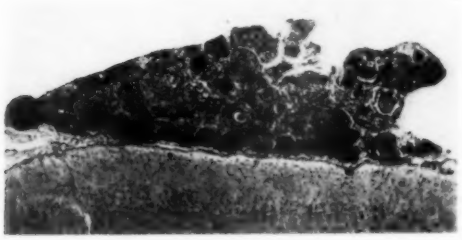

FIG. 9.

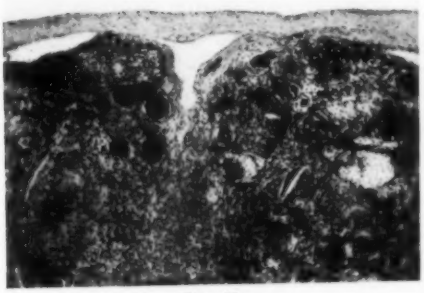

FIG. 10.

FIG. 11.

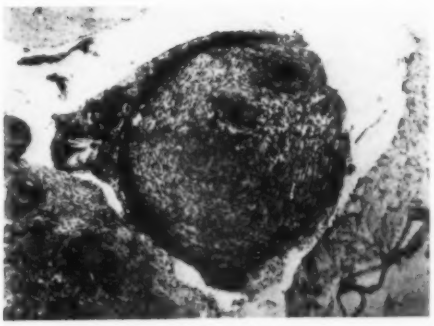

FIG. 12.

A NOTE UPON THE OCCURRENCE OF CONGENITAL ATRIO-VENTRICULAR DISSOCIATION

REPORT OF A CASE OF CONGENITAL COMPLETE HEART BLOCK

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It is an extremely interesting fact that although the number of cases of heart block, either congenital or acquired, occurring in childhood reported in the literature are comparatively few, the authentic instances of congenital partial or complete atrio-ventricular dissociation, in which the diagnosis has been established by graphic records, number but seven. All of these, with the single exception of the case reported by d'Espine,[1] have been found in association with congenital malformation of the heart. It is thus apparent that in spite of the relatively high incidence of congenital heart disease the occurrence of defects involving the conduction system and the presence of heart block associated with congenital malformation are infrequently met with.

In determining the exact etiology of the bundle defect in cases of heart block of assumed congenital origin, it is not within the limits of absolute accuracy to state positively that the lesion present existed at birth, but in the absence of any history of a possible post-natal infection and with the evidence of clinical congenital heart disease such a conclusion seems warranted. In this connection it is well to remember that it is extremely rare to find pathological changes dependent upon post-natal infections before the fourth or fifth year, and that the three great sources of endocardial and myocardial disease in childhood are, in the order of their gravity, rheumatic fever, scarlet fever and diphtheria; the first two involving both the valves and myocardium, the latter essentially the myocardium alone, so that in the absence of any history of these infections in the presence of cardiac disease, at least up to the age of four or five, the assumption of the congenital nature of the disease is wholly justifiable. It should further be emphasized that all evidences of cyanosis and clubbing of the fingers may be absent even with conspicuous congenital lesions.

Because of the uncertainty of the diagnosis in the absence of graphic records we have not included with the seven cases referred to above five cases of atrio-ventricular block occurring in childhood probably congenital in origin but not definitely established as such; two reported by Morquio,[2] one by von Starck,[3] one by Gill [4] and one by Zahorsky.[5] The case reported by Gill in a child of six, and regarded by him as a congenital heart block, had a ventricular rate varying between 47 and 60, and though the report is accompanied with respiratory and radial tracings the absolute confirmation of a radial and venous tracing is lacking.

In their article upon heart block in children Eyster and Middleton [6] tabulated the cases previously reported, with the exception of Gill's, including both congenital and acquired, adding a case of their own. In this series these writers have included the case reported by van den Heuvel,[7] which we include in the seven cases of congenital heart block referred to above, but to which it is possible that exception might be taken on the basis of a strict interpretation of our title. The individual the subject of v. d. Heuvel's report was 23 years of age when first seen by himself and it was not until her 15th year that the slow cardiac rate was first noticed by a physician, though the history of the syncopal attacks dated from her second year. In view, however, of the detailed history as given, there can be no doubt, it seems to us, as to the congenital nature of the lesion in this case.

Upon the same ground of advanced age and in the entire absence of any definitely known early data, though with no history of rheumatic fever, chorea, scarlet fever or diphtheria, we have not included with the authentic cases the instance of complete atrio-ventricular dissociation associated with congenital pulmonary stenosis in a woman of 27 reported by one of us in an earlier communication.[8]

The previously reported cases of congenital atrio-ventricular dissociation, in which the diagnosis has been confirmed by graphic records, may be summarized briefly as follows. The case recorded by Fulton, Judson and Norris [9] in a child aged 22 months, with a ventricular rate of 42 to the minute, first noticed at the age of seven days, with a hypertrophied heart and a loud systolic murmur, from whom the polygraphic tracings showed an undoubted complete heart block. Wipham's [10] two cases, one baby of 18 months with a ventricular rate of 56 to 64, with a slight cardiac hypertrophy and a loud systolic murmur interpreted as due to a patent interventricular septum, from whom the electrocardiographic records showed a 2:1 block. The second case, a child of 12 years with a ventricular rate varying between 40 and 64, with an enlarged heart and a systolic murmur of maximum intensity in the fourth interspace near the sternum, with no cyanosis or clubbing of the fingers, from whom the electrocardiographic tracings showed an a-v block varying from 2:1 to a 3:1 rhythm with at times a complete dissociation.

The first of these children was admitted to the hospital suffering from whooping cough and bronchitis, and later developed a nasal discharge that proved to be of a "diphtheritic nature." In spite of this factor in the history the early clinical evidence of a very slow heart rate and the evidence of congenital heart disease would seem to justify the conclusion as to the congenital nature of the lesion. As to the second case, there may well be a reasonable doubt concerning the etiology of the

lesion since the slow heart rate was not noticed until the sixth year and there is the further history of an attack of measles "at an early age"; the exact date is not given. On the other hand, the clinical evidence of congenital heart disease, with the absence of all history of any illness but the attack of measles, favors the view that the cardiac lesion was congenital.

D'Espine (*loc. cit.*) has reported an instance of complete heart block in a boy of eight, regarded as undoubtedly congenital, and characterized by a single syncopal attack, with a slightly hypertrophied heart but no cyanosis or clubbing of the fingers, in whom there was present a soft systolic murmur interpreted as of extra-cardiac origin. This is the single instance of heart block in the literature believed to be not dependent upon acquired cardiac disease and not associated with congenital malformation of the heart. It is interesting that the child had a positive Wassermann reaction but no stigmata of

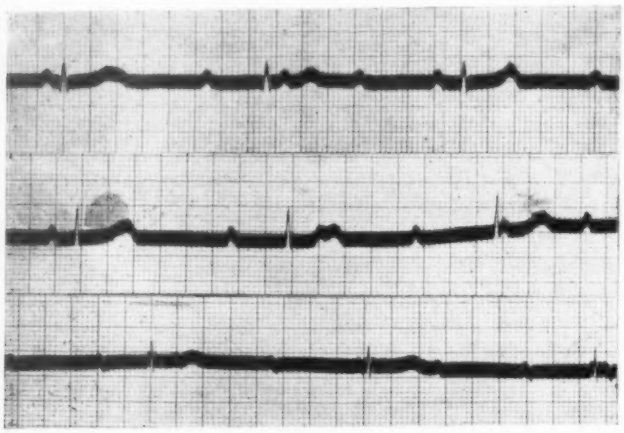

FIG. 1.—May 25, 1919. Complete atrio-ventricular dissociation. Atrial rate 103, ventricular rate 37. Note the absence of any evidence of ventricular preponderance.

hereditary syphilis. Graphic records showed a complete a-v dissociation, with an auricular rate of 104 and a ventricular rate of 26 to the minute, unaltered by atropine.

The case reported by van den Heuvel (*loc. cit.*) in a woman of 23, which in view of its long duration and the clinical history of repeated syncopal attacks is of unusual interest. At birth the child is said to have been conspicuously cyanotic, but apparently nothing abnormal was noted until her second year when the first syncopal attack occurred, lasting but a few moments. Following this she remained well until her sixth year, during which she had three syncopal attacks. There is a history of measles at eight, following which syncopal attacks again occurred. She then remained well until her 15th year. Similar syncopal attacks occurred in her 18th, 20th and 22d years, characterized by loss of consciousness and cyanosis but without convulsions. There was slight cyanosis of the extremities, an enlarged heart with a ventricular rate of 34 to the minute and a harsh systolic murmur of maximum intensity in the third left interspace. The clinical diagnosis was patent interventricular septum with slight pulmonary stenosis. Graphic records showed a complete a-v dissociation.

The case reported by Bass [11] was in a boy first seen at the age of 15 years and who also had a history of a mild attack of pneumonia at three and measles at five, in whom the slow cardiac rate, 37 to the minute, was first noticed at the age of 13. There was a slightly enlarged heart with a short blowing systolic murmur at the apex and a very rough systolic murmur of maximum intensity over the third left interspace and transmitted downwards, for a short distance, but not upwards. The lesion was interpreted as pulmonary stenosis. There was no cyanosis and no clubbing of the extremities. Electrocardiographic tracings showed the presence of an alternating 2 : 1 rhythm with a complete dissociation.

Finally the case reported by Rosenson [12] in a girl of 10 years who first came under observation at the age of eight, with a cardiac rate of 44 and without any other symptoms than a complaint of slight pain and distress over the precordium following exertion. There was no cyanosis. The heart was slightly enlarged. There was a long sawing diastolic murmur heard over the precordium, of maximum intensity in the second left interspace. The murmur was considered to be due to a congenital malformation because of its character and the history of the case. The defect was believed to be a direct communication between the aorta and pulmonary arteries just above the aortic valves. Electrocardiographic tracings—no figures are published—showed a complete a-v dissociation.

To the above cases we wish to add the following instance of congenital complete atrio-ventricular dissociation occurring in a girl in whom the exact diagnosis made at the age of three has been repeatedly confirmed by graphic records.

CLINICAL REPORT

K. W. Born Nov. 16, 1909. First seen March 11, 1915. Aged 5 years. Diagnosis: congenital malformation of the heart and congenital heart block.

F. H.—Negative.

P. H.—Third of four children, others well. Weight at birth between 9 and 12 pounds. Nursed six months and did well. Mother thinks that she had rather a blue look around the mouth and had a blue vein very prominent across the nose. At nine months she had bronchitis and it was noticed at this time that she had a cardiac murmur. At about two years of age she was seen by Dr. Walter James, who also confirmed the presence of a murmur, and a year later, when she was three, he saw her again and had electrocardiographic records taken which showed the presence of a complete heart block. In the summer of 1914 she had a slight syncopal attack and was seen by Dr. Gannett, of Boston, who also confirmed the diagnosis of heart block. She has never been short of breath, never cyanotic and has never had any evidences of an impaired myocardium. Recently she has played about just as any other child would do and there has been no more shortness of breath or cyanosis than would be seen with a perfectly normal child.

S. P.—The child is moderately nourished, rather spare and of good color. There is no cyanosis, dyspnœa, changes in the gums or enlarged viscera.

Heart.—The apex beat is in the fifth interspace 8.5 cm. to the left of the mid-sternal line. Very slightly enlarged to the left. Rate very slow, 23 to the half minute. The sounds are clear, except for a rather rough and harsh systolic murmur of maximum intensity near the apex, not transmitted. The third heart sound is very plain. It is obvious from the venous pulsations

in the neck that there are two or three times as many auricular as there are ventricular systoles. The pulse is of good quality, regular and very slow. Electrocardiographic records were taken and the diagnosis of complete atrio-ventricular dissociation confirmed. Roentgenograms were also taken.

Seen again January 29, 1916. She had pertussis from June until September, 1915. Since then she has been very well. She has grown and gained in weight. Weight now 46 pounds and 6 ounces. Color good. She does not get short of breath even when running, but complains of an occasional "stitch in her side." Pulse 44 to the minute. Apex beat as noted above. Heart very slightly enlarged to the right. There is a harsh but somewhat blowing murmur which is heard over the precordium from the apex nearly to the base, but further to the left than is usually the case with open interventricular septal murmurs. The second pulmonic sound is accentuated. No auricular sounds heard. No pulmonary signs.

Seen March 6, 1917. Weight 54 pounds and 12 ounces. Height 50¼ inches. She has been very well. No illness of any kind except a mild attack of otitis media which healed promptly after incisión. No dyspnœa. No cynanosis. The murmur is certainly much fainter than formerly, can hardly be heard when the child is standing or sitting up. It becomes more audible when she is lying down. Cardiac outline as previously noted. Ventricular rate 48 to the minute.

Seen May 25, 1919. She has developed very well. Present weight 74 pounds and 10 ounces. Height 4 feet 7 inches. Her color is good. She is able to skate 20 blocks on roller skates without any difficulty or distress. Occasionally she has what is called a "tired day" when she is glad to stay quiet, but these are infrequent. The apex beat is in the fifth interspace 8.5 cm. from the mid-sternal line. There is rather a rough short systolic murmur heard all over the precordium. The first sound is usually not very loud. From time to time, however, there is a marked accentuation of this sound. The heart rate is slow, 48 to 52 to the minute. From time to time a very characteristic third heart sound is audible. The cardiac outline seems no larger relatively than it was before. Electrocardiographic tracings taken at this time show a complete atrio-ventricular dissociation. Auricular rate 103, ventricular rate 37 to the minute. (See Fig. 1.)

The following interesting points brought out by this history may be emphasized briefly: The early diagnosis of the cardiac murmur at nine months of age, an evidence of the existence of congenital malformation of the heart, and the confirmation of the clinical diagnosis of heart block by electrocardiographic records at the age of three, the history of having passed successfully through an attack of pertussis lasting four months in 1915, and the child's present normal appearance and ability to play and romp as any normal child.

When last seen in May, 1919, the very striking occasional accentuation of the first sound of the heart was a conspicuous feature and was interpreted as being due to the synchronous systole of auricle and ventricle, although the auricular systole falling in the long diastolic pauses could not be made out.

Perhaps the most significant feature of this small series of cases is the absence of any history of grave syncopal attacks except in the instance reported by v. d. Heuvel, which with one exception had the lowest ventricular rate, a fact in strict accordance with the physiology of the idio-ventricular rhythm. In cases 4 and 8 of our table there was a history of one syncopal attack of very mild type. It would seem apparently that as a result of the congenital nature of the lesion the heart early acquired the power of adapting itself to the constant load necessary to maintain an efficient circulation.

It is greatly to be regretted that we were unable to carry out an observation of the response to atropine, but in view of the long duration of the definitely known block there can be no question as to its organic nature. After exercise and under vagus stimulation there was no appreciable change in the ventricular rate recorded by the galvanometer.

Embryological Development

Considering the nature of the development defect, it is apparent that the lesions associated with disturbances of conduction are, in the recorded cases, entirely confined to defects involving the main stem rather than one of its branches. It is also evident that the structural malformation involving the proximal region of the main bundle must occur at a very early period of embryonic development, possibly at that time when the first evaginations of the interventricular cavities take place at about the fourth week after fertilization. We know that the evolution of the His-Tawara bundle results from the growth downwards of the primitive cardiac tube, forming the ventricular cavities and interventricular septum, gradually severing all muscular continuity between auricles and ventricles,

TABULAR REVIEW OF CASES

Case No.	Author	Sex	Age	Age at time of first observation of slow cardiac rate	Hypertrophy of the heart	Interpretation of the cardiac murmur	Nature of the A-V dissociation	Ventricular rate	History of syncopal attacks	Presence or absence of cyanosis	Presence or absence of clubbing of extremities	Symptoms complained of
1.	Fulton, Judson and Norris.	M.	22 months.	7 days.	Slight.	Patent interventricular septum.	Complete.	42	None.	Absent.	Absent.	None.
2.	Wipham.	F.	18 months.	18 months.	Slight.	Patent interventricular septum.	Partial 2:1 rhythm.	56-64	None.	Absent.	Absent.	None.
3.	Wipham.	F.	12 years.	6 years.	Marked.	Patent interventricular septum.	Complete.	40-64	None.	Absent.	Absent.	Pain around the heart on exertion.
4.	d'Espine.	M.	8 years.	8 years.	Slight.	Extra cardiac.	Complete.	20-32	One attack.	Absent.	Absent.	None.
5.	van den Heuvel.	F.	23 years.	15 years.	Marked.	Patent interventricular septum.	Complete.	34	Present.	Slight.	Absent.	Syncopal attacks.
6.	Bass.	M.	15 years.	13 years.	Slight.	Not definitely stated.	2:1 and complete.	37	None.	Absent.	Absent.	Dyspnœa and palpitation on exertion.
7.	Rosenson.	F.	10 years.	8 years.	Marked.	Communication between aorta and pulmonary artery just above aortic valves.	Complete.	44	None.	Absent.	Absent.	Pain and distress around the heart on exertion.
8.	Present case.	F.	10 years.	3 years.	Slight.	Patent interventricular septum.	Complete.	37	One attack at 4 years.	Absent.	Absent.	Occasional "stitch in the side."

except for a narrow strand that persists and is later differentiated as the atrio-ventricular bundle. It is readily conceivable that abnormal development at this time may involve only that group of cells which later is to become the a-v node or upper part of the main stem to a widely varying degree.

In view of the definite relationship existing between the main bundle of the conduction system and the interventricular foramen as shown by Keith,[13] is is interesting that more frequent disturbances of the conduction system are not met with. But, as pointed out by Keith, the a-v bundle is developed in the *upper* margin of the septum which represents a part of the lumen of the primitive heart. May not this fact explain its frequent escape from injury in these cases of congenital patent interventricular septum?

SUMMARY

To the seven definitely proven instances of congenital malformation of the conduction system of the heart we add another, making eight such cases in which the diagnosis has been confirmed by graphic records.

We have been unable to find any report of a histological study of any of these cases.

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ONE FACTOR IN THE MECHANISM OF HEMOLYSIS IN HEMOLYTIC ANEMIA

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The different types of anemia fall into three general groups; those due to blood loss, those due to faulty production of red blood cells either quantitative or qualitative, and those due primarily to increased destruction of the red blood cells in the body. This study is concerned with the anemias of the last group, the so-called hemolytic anemias, seen characteristically in two clinical conditions, primary pernicious or Addisonian anemia and hemolytic icterus.

The causes of the abnormal destruction of red blood cells in the hemolytic anemias are not yet established. The studies of Faust and Tallqvist on the anemia of *Dibothriocephalus latus* infestation,[1] of Iwao on the results of p-oxy-phenylethylamin (or tyramin) injection,[2] of Bunting[3] and of others, afford evidence that a blood picture such as is seen in Addisonian anemia may result from the action of a chemical hemolysin in the body. In the so-called hemolytic anemias efforts to prove the presence of a foreign hemolytic agent have not been successful. This evidence, however, is not necessary to prove the hemolytic character of the hemolytic anemias. If our understanding of fat digestion and absorption is correct, there is normally in the blood a sufficient amount of powerful hemolytic agents in the form of soluble soaps to explain the phenomena observed, provided they are free to act as hemolysins. Studies on these normal circulating hemolysins to determine whether they are increased in amount or altered in form in the hemolytic anemias have not been conclusive.[4, 5, 6, 7] If the hemolysis in the hemolytic anemias is not due (1) to the presence of a foreign hemolysin or (2) to an increase in the hemolysins normally present, another possibility that suggests itself is (3) some disorder in the mechanism that normally prevents the hemolytic substances present in normal individuals from acting as hemolysins.

It has not been definitely established why the hemolytic agents present in normal blood do not hemolyse the red blood cells; but it has been shown repeatedly that serum has great power to protect washed red blood cells from hemolysis by many different agents. Thus Ford[8] demonstrated protective power in normal serum against hemolysis by the hemolytic ingredient of *Amanita phalloides*, Moss[9] against the isohemolysins of the human species, Sellards[10] against the hemolysis of bile and bile salts, and Moss and Barnes[11] against cobra venom hemolysis. Noguchi[12] and Liebermann[13] showed that normal serum had power to protect against hemolysis by sodium oleate, and later Lamar proved this protective power not only against hemolysis of red blood cells[14] but also against lysis of pneumococci.[15] In view of these facts it seems reasonable to suppose that the hemolytic agents present in the blood normally do not hemolyse because of the power of serum to protect red blood cells against hemolysis by such chemical agents. If for any reason this protective power against hemolysis were diminished the normal hemolytic substances present in normal amounts might act as hemolysins in the body. It is suggested, therefore, that the hemolysis occurring in the hemolytic anemias, since not proven to be due to the presence of a foreign

hemolysin or to an increase of hemolysins normally present, may be dependent upon a diminution of the protective power of the serum against hemolytic agents. To test this hypothesis experiments were undertaken to determine the degree of protective power against hemolysis exhibited by the serum of patients having a hemolytic anemia, of patients with other diseases, and of normal persons.

To study the protective power of serum, a series of test-tubes containing constant quantities of different dilutions of hemolytic agent was set up and a constant quantity of the serum to be tested was added to each of these tubes. This mixture was incubated and cooled, a constant quantity of guinea-pig corpuscles was added and incubation was continued. Each tube of the series in the titration contained:

(1) Desired dilution of hemolytic agent.....2.00 c. c.
(2) Serum to be tested (diluted 1-20).....0.25 c. c.
(3) Washed guinea-pig cells (7.5 per cent suspension) .....0.25 c. c.

Total mixture .....2.50 c. c.

For the hemolytic agent sodium oleate—"Merck's highest purity"—was chosen because it is readily soluble, is a powerful hemolytic agent, and one that may be supposed to be present more or less constantly in the body. Throughout all the experiments to be reported the sodium oleate came from the same two ounce bottle in which it was delivered. The preparation is a white powder. Of this 100 mgm. were dissolved in 100 c. c. of sterile normal salt solution and further dilutions were made from this 1 to 1000 stock solution. The solution when used was slightly opaque, this opacity becoming greater on standing and with manipulation. The hemolytic power of the soap solution diminishes with the increase in opacity. To ensure the same degree of hemolytic power of the soap solution in each titration the technique used in its preparation was always uniform. The soap powder was dissolved in the salt solution each afternoon for the titrations of the next day, the sterile container was stoppered with cotton and, to ensure uniform conditions, the solution was kept in the incubator over night. The further dilutions made from this 1 to 1000 sodium oleate solution were carried out just before setting up the titration. It was diluted with sterile normal salt solution according to Table 1 in such a manner that the dilutions obtained were four-fifths of the dilution desired in the final preparations.

Two cubic centimeters of each dilution were put in a separate one of a series of tubes. When the serum and guinea-pig corpuscles had been added to each tube the total volume was 2.5 c. c., hence the necessity of making dilutions of sodium oleate only four-fifths of the desired final dilution.

The patient whose serum was to be tested was bled either late the afternoon before or on the morning of the titration. To ensure serum free from hemoglobin the syringe and needle with which the venapuncture was done were washed with sterile normal salt solution after they had been boiled. The blood (1 to 2 c. c.) was introduced into a sterile test-tube, allowed to clot and within two hours of the venapuncture the clot was separated, the tube centrifuged and the serum removed with a pipette. This was then immediately inactivated on the water-bath at 56° C. for one hour. If not inactivated, serum rapidly loses its protective power; if inactivated the protective power diminishes somewhat more slowly. Serum retains some protective power for a long time apparently, for one specimen six weeks old and covered with mould still showed some power to protect guinea-pig cells against hemolysis by sodium oleate. The variation in this protective power of serum on standing was very irregular,

TABLE I

TO MAKE DILUTIONS OF SODIUM OLEATE

c. c. of 1-1000 sodium oleate solution	+	c. c. normal salt solution	=	Dilution r sulting	giving	Dilution of sod. oleate in final preparation
.1		2.3		24,000		30,000
.1		2.7		28,000		35,000
.1		3.1		32,000		40,000
.1		3.5		36,000		45,000
.1		3.9		40,000		50,000
.1		4.3		44,000		55,000
.1		4.7		48,000		60,000
.1		5.1		52,000		65,000
.1		5.5		56,000		70,000
.1		5.9		60,000		75,000
.1		6.3		64,000		80,000
.1		6.7		68,000		85,000
.1		7.1		72,000		90,000
.1		7.5		76,000		95,000
.1		7.9		80,000		100,000
.1		8.3		84,000		105,000
.1		8.7		88,000		110,000
.1		9.1		92,000		115,000
.1		9.5		96,000		120,000
.1		9.9		100,000		125,000
.1		10.3		104,000		130,000
.1		10.7		108,000		135,000
.1		11.1		112,000		140,000
.1		11.5		116,000		145,000
.1		11.9		120,000		150,000
.1		12.3		124,000		155,000
.1		12.7		128,000		160,000
.1		13.1		132,000		165,000
.1		13.5		136,000		170,000
.1		13.9		140,000		175,000
.1		14.3		144,000		180,000
.1		14.7		148,000		185,000
.1		15.1		152,000		190,000
.1		15.5		156,000		195,000
.1		15.9		160,000		200,000

however, so in the titrations recorded the serum, when used, was always less than twenty-four hours old. If the venapuncture was done the afternoon before, the inactivated serum in a sterile, stoppered test-tube was kept in the ice-box over night. Just before the titration was set up, sufficient normal salt solution was added to the inactivated serum to make a 1 to 20 dilution. Of this 0.25 c. c. was added to each tube, thus diluting the serum ten times more (the final volume of fluid in each tube was 2.5 c. c.) so tha in the final preparation the serum was present in a 1 to 200 concentration.

The suspension of guinea-pig cells used was always freshly prepared, because the resistance of guinea-pig cells to hemol-

ysis is markedly diminished on standing any length of time in salt solution. The morning of the titration guinea-pig blood was taken by cardiopuncture into a syringe washed out with 1.5 per cent citrate in normal salt solution after boiling, and containing one or two cubic centimeters of the same citrate solution. This blood was introduced into a sterile centrifuge tube containing sufficient citrate solution to prevent clotting, the cells were thrown down by centrifugation and washed three times in normal salt solution. With each preparation, the last time the cells were centrifuged, the process was continued at the same speed for exactly twenty minutes, the volume of cells read and salt solution equivalent to one third the volume of cells was added to them giving a 75 per cent suspension of cells. Immediately before the cells were added to the tubes of the titration, this 75 per cent suspension was diluted ten times and 0.25 c. c. of the resulting 7.5 per cent suspension was added to each tube, thus giving a 0.75 per cent concentration of cells in the final preparation.

The 0.25 c. c. of serum 1 to 20 was added to the tubes containing 2 c. c. of different sodium oleate dilutions and this was incubated in a water-bath for one-half hour at 37.5° C. and then allowed to cool at room temperature for the same length of time before the guinea-pig corpuscles were added. It will be noticed that during this time the serum was allowed to act on the hemolytic agent before the corpuscles were added, the sodium oleate dilutions were only nine-tenths of the dilutions recorded in the final preparation for each tube, and the serum was not 1 to 200 but 1 to 180. After this preliminary incubation and cooling of the serum plus sodium oleate, the guinea-pig corpuscles were added and incubation was continued in the water-bath at 37.5° C. for two hours. Every half hour during this period each separate tube was shaken gently but enough to ensure an even suspension of the corpuscles, and a reading of the degree of hemolysis was made. At the end of two hours the test-tube racks were removed from the water-bath, each tube was shaken again and the racks put in the ice-box for one hour. This permitted enough sedimentation to make a final reading of the presence and amount of hemolysis in each tube. The recorded results then appear as in Table II. The interaction of serum, sodium oleate and corpuscles may be greatly hastened or increased by a rise in temperature, or retarded by a fall; hence, to get comparative results it is necessary that the incubation be at the same temperature (37.5° C.) each time. Furthermore, the hemolysis will continue for many hours even in the ice-box, so the time at which the readings were made was constant. Hemolysis progressed so slowly at lower temperatures, however, that rarely after one hour only in the ice-box did it go beyond that seen after two hours' incubation; but the one hour permitted sedimentation and thus facilitated readings of the results. With some practice, however, accurate reading of hemolysis can be made without sedimentation. The tubes may not be centrifuged, for the corpuscles are so injured by the exposure to sodium oleate solutions that the trauma incident to centrifugation produces hemolysis even in the higher dilutions of

TABLE II

TITRATION OF THE SERA OF SIX CHARACTERISTIC CASES FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SODIUM OLEATE SOLUTION

F. E. *Normal Control.*

Dilutions of sod. oleate	45,000	50,000	55,000	60,000	65,000	70,000	75,000	80,000	85,000	90,000	95,000	Control
Incubation ½ hr.	−	−	−	−	−	−	−	−	−	−	−	−
" 1 hr.	+−	+−	−	−	−	−	−	−	−	−	−	−
" 1½ hrs.	+−	+−	−	−	−	−	−	−	−	−	−	−
" 2 hrs.	+−	+−	−	−	−	−	−	−	−	−	−	−
One additional hr. in ice-box..	+−	+−	−	−	−	−	−	−	−	−	−	−

R. K. Age 58. *Diabetes mellitus.* R. B. C. 5,064,000, Hb. 95%, W. B. C. 10,000, P. M. N. 61%.

Incubation ½ hr.	−	−	−	−	−	−	−	−	−	−	−	−
" 1 hr.	−	−	−	−	−	−	−	−	−	−	−	−
" 1½ hrs.	+=	−	−	−	−	−	−	−	−	−	−	−
" 2 hrs.	+−	+−	−	−	−	−	−	−	−	−	−	−
One additional hr. in ice-box..	+	+−	−	−	−	−	−	−	−	−	−	−

B. R. Age 29. *Typhoid fever.* R. B. C. 2,616,000, Hb. 55%, W. B. C. 5,600, P. M. N. 80%.

Incubation ½ hr.	−	−	−	−	−	−	−	−	−	−	−	−
" 1 hr.	+	+−	−	−	−	−	−	−	−	−	−	−
" 1½ hrs.	+	+−	+−	+−	+−	−	−	−	−	−	−	−
" 2 hrs.	+	+−	+−	+−	+-	+−	−	−	−	−	−	−
One additional hr. in ice-box..	+	+−	+−	+−	+−	+−	−	−	−	−	−	−

W. J. M. Age 50. *Carcinoma of bladder.* R. B. C. 1,896,000, Hb. 28%, W. B. C. 23,700.

Incubation ½ hr.	−	−	−	−	−	−	−	−	−	−	−	−
" 1 hr.	+−	+−	−	−	−	−	−	−	−	−	−	−
" 1½ hrs.	+−	+−	−	−	−	−	−	−	−	−	−	−
" 2 hrs.	+	+	+−	+-	−	−	−	−	−	−	−	−
One additional hr. in ice-box..	+	+	+−	+−	−	−	−	−	−	−	−	−

T. C. Age 59. *Pernicious anemia.* R. B. C. 1,584,000, Hb. 34%, W. B. C. 4,600, P. M. N. 56%.

Incubation ½ hr.	+=	+=	+=	−	−	−	−	−	−	−	−	−
" 1 hr.	+−	+−	+−	+=	+=	−	−	−	−	−	−	−
" 1½ hrs.	+	+	+	+−	+−	+−	+−	+=	−	−	−	−
" 2 hrs.	+	+	+	+	+	+−	+−	+−	−	−	−	−
One additional hr. in ice-box..	+	+	+	+	+	+−	+−	+−	+−	+−	−	−

D. W. Age 35. *Pernicious anemia.* R. B. C. 1,470 000, Hb. 43%, W. B. C. 4,800, P. M. N. 61%.

Incubation ½ hr.	−	−	−	−	−	−	−	−	−	−	−	−
" 1 hr.	+−	+−	+−	−	−	−	−	−	−	−	−	−
" 1½ hrs.	+	+	+−	+−	+−	−	−	−	−	−	−	−
" 2 hrs.	+	+	+	+−	+−	+−	+=	+=	+=	−	−	−
One additional hr. in ice-box..	+	+	+	+−	+−	+−	+−	+−	+−	+−	−	−

Symbols: (−) indicates no hemolysis.
(+=) " beginning hemolysis.
(+−) " partial hemolysis.
(++−) " hemolysis almost complete.
(+) " complete hemolysis.

In the last column is the control tube of serum 1-200 and guinea-pig cell suspension 0.75% in normal salt solution.

the sodium oleate, when in much stronger solutions after sedimentation without trauma in the ice-box there is no hemolysis.

With each titration two controls were set up.

(1) A 0.75 per cent suspension of guinea-pig cells in the normal salt solution used was incubated and examined for hemolysis at the same time as the tubes containing both serum and hemolytic agent. This was always negative.

(2) With each serum tested a tube was set up containing the same amount of serum and guinea-pig cells as the tubes of the titration but containing no hemolytic agent, the contents of the tube being made up to the required volume, 2.5 c. c., with sterile normal salt solution. If the serum was inactivated this was always negative for hemolysis. Occasionally, however, if such a preparation was set up with serum that had not been inactivated, it showed a slight amount of hemolysis.

Having thus proved (1) that the guinea-pig cells and normal salt solution used and (2) that the guinea-pig cells and salt solution plus the serum to be tested, showed no hemolysis, it remained to be proved that the different degrees of hemolysis recorded depended on the amount of protective power of the serum and not on variations in either the hemolytic power of the sodium oleate or the resistance of the guinea-pig cells.

When experiments were undertaken to determine whether or not the sodium oleate solutions as made up from day to day exhibited a constant degree of hemolytic power, the preparations were set up in exactly the same way as in titrations for protective power of serum except that human instead of guinea-pig corpuscles were used and no serum was added, the contents of the tubes being made up to the required volume (2.5 c. c.) with sterile normal salt solution. A slight variation in results was found when corpuscles of different individuals were used.

November 14	Highest dilution showing hemolysis
(1) F. E., normal	70,000
(2) C. G., normal	65,000

This finding, however, apparently is due to differences in resistance to hemolysis of cells of different persons, for titrations of cells of one person set up in triplicate on the same day all showed the same reading and when repeated on subsequent days with fresh sodium oleate solutions the same results were obtained.

(1) F. E. normal

		Highest dilution showing hemolysis
November 14		70,000
November 15	(1)	70,000
	(2)	70,000
November 17	(1)	70,000
	(2)	70,000
	(3)	70,000

(2) C. G. normal

		Highest dilution showing hemolysis
November 14		65,000
November 18	(1)	65,000
	(2)	65,000
	(3)	65,000
November 19	(1)	65,000
	(2)	65,000
	(3)	65,000

(3) F. C. normal

		Highest dilution showing hemolysis
November 5		70,000
November 18	(1)	65,000
	(2)	65,000
	(3)	65,000
November 19	(1)	65,000
	(2)	65,000
	(3)	65,000

The findings in the last case, F. C., which showed different figures for a titration on November 5 and those on November 18 and 19 suggest that in this instance there might have been some variation in the sodium oleate solution; but the constant findings in titrations on nearby days point more strongly to the possibility that the difference is due to a variation from time to time in the resistance of one person's corpuscles to sodium oleate hemolysis.

When the constancy of the sodium oleate solutions had been established, the resistance of guinea-pig cells to hemolysis was studied in exactly the same way. Guinea-pig cells are much less resistant to sodium oleate hemolysis than human corpuscles and considerable variation was found. Hemolysis occurred up to dilutions of 1-140,000 to 1-180,000. This wide variation in resistance of guinea-pig cells, unprotected by serum, to hemolysis by sodium oleate solutions occurred in cells taken from the same pig on different days. Thus, for the cells of the same pig:

Titration of	Highest dilution showing hemolysis
Dec. 8, 1919	140,000
Dec. 10, 1919	170,000

This was not due to repeated bleeding, because pigs bled for the first time or for several days in succession might show cells of low resistance; thus:

Cells of	Highest dilution showing hemolysis
Pig bled three days in succession	170,000
Pig bled for the first time	170,000

Or they might show a greater resistance; thus:

Cells of	Highest dilution showing hemolysis
Pig bled four days in succession	145,000
Pig bled two days in succession	155,000

The difference in the resistance of the guinea-pig cells to hemolytic agents is not so great, however, as these titrations would indicate. Two suspensions of the cells with widely different figures for the highest dilution showing a trace of hemolysis might have the highest dilution showing complete hemolysis the same. Thus:

Highest dilution showing	some hemolysis	complete hemolysis
(1)	170,000	110,000
(2)	140,000	110,000

It is further of interest that in the dilution 1 to 140,000 there may not be any more hemolysis in a suspension of cells showing some hemolysis to a dilution of 1 to 170,000 than in one in which this dilution, 1 to 140,000, was the highest in which hemolysis occurred. At any rate it is evident that the difference in resistance of the guinea-pig cells to sodium oleate solution was not great enough to influence the reading of the serum test as set up, for with cells from different guinea-pigs

showing a variation when unprotected by serum the same results were obtained when protected by the same serum.

Patient	Last tube showing hemolysis 4 times bled pig	twice bled pig
Normal	55,000	55,000
Hemolytic icterus	75,000	75,000
Pernicious anemia convalescent	75,000	75,000

And again, the constant results obtained with known normal serum not only bears evidence of the constant grade of hemolytic power exhibited by the sodium oleate solution, but also of the regularity with which cells of different guinea-pigs act when protected by the same serum.

F. E. normal control	Titration of	Highest dilution showing hemolysis
	January 13, 1920	55,000
	February 21, 1920	55,000
	March 8, 1920	55,000
	March 22, 1920	55,000

Sodium oleate solution is a colloid and when it is used as the hemolytic agent reliable results in quantitative hemolytic experiments are difficult to obtain. In spite of the most extreme care always to use a uniform technique, irregularities were sometimes encountered which were difficult to explain. On one occasion no satisfactory titrations could be obtained over a period of two weeks. For two weeks previous to this the sodium oleate powder had been exposed inadvertently to the fumes of petroleum ether, but after one week in a different cupboard it again reacted satisfactorily. This may have been merely a coincidence. Variations in humidity seemed to influence the properties of sodium oleate solutions, for in very wet weather the preparations were somewhat more opaque in appearance and such solutions seemed to suffer a slight but definite diminution in hemolytic power as compared with the clearer solutions which were the rule at ordinary times. Because of such difficulties extremely critical examination of results was necessary. It was for this reason that readings on each series of titrations were recorded at four half-hour periods as tabulated in Table II. If there was any irregularity in the progression of hemolysis from one reading to another it was considered sufficient evidence to condemn the entire titration. Similarly, if the known normal serum included as a control in each day's series of titrations of unknown sera did not fall within the narrow limits established by titration of the serum of many normal persons, the results of the entire series were not accepted; and if the other controls indicated that there was something wrong with either the sodium oleate solution, the serum, or the suspension of guinea-pig cells, all the titrations of that day's series were repeated with fresh preparations.

Throughout the experiments recorded the glassware used was chemically clean and sterile. Sterile technique was observed except that no effort was made to sterilize the sodium oleate powder and after it was dissolved in the sterile normal salt solution the mixture was not autoclaved. After a trial of several methods of cleaning glassware that found most satisfactory for this work was suggested by Dr. W. M. Allen working in this laboratory. After use the glassware was washed several times in tap-water, no soap being used. It was then immersed in weak ammonium hydrate for twelve or more hours, removed from this and washed thoroughly several times in hot tap-water. This was followed by washing in distilled water, after which it was dried at a low temperature in the hot air sterilizer with the door open. After cooling, the glassware was stoppered with cotton and sterilized in the dry sterilizer, care being taken to avoid charring of the cotton stoppers. Quantitative measurements were made with volumetric pipettes and for the finer dilutions non-certified 1 c. c. pipettes graduated in hundredths were used. The titrations were set up in ordinary Wassermann test-tubes, 10 x 100 millimeters.

Titrations of a constant quantity of human serum as outlined above against varying strengths of sodium oleate solution, guinea-pig corpuscles being used as indicator, produced interesting results. From an examination of Table III it

TABLE III

RESULTS OF TITRATIONS OF SERUM OF NORMAL PERSONS FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SODIUM OLEATE

No.	Name	Blood group	Date	Highest dilution of sod. oleate showing hemolysis
1	K. M. D.	I	11/5/19	40,000
2	H. P.	II	7/25/19	50,000
3	A. L.	II	8/12/19	55,000
4	V. R. M.	II	8/14/19	50,000
5	F. D. C.	II	10/29/19	45,000
6	C. G. G.	II	10/29/19	45,000
7	C. S.	II	1/14/20	50,000
8	H. R. B.	II	3/2/20	55,000
9	C. H. C.	III	8/13/19	50,000
10	H. M.	IV	8/8/19	50,000
			10/8/19	55,000
11	H. M. C.	IV	9/24/19	50,000
12	D. M. S.	IV	10/31/19	50,000
13	E. L.	IV	1/16/20	55,000
14	W. M. A.	IV	1/19/20	50,000
15	F. A. E.	IV	8/6/19	55,000
			9/18/19	40,000
			10/16/19	40,000
			10/24/19	45,000
			12/11/19	45,000
			12/15/19	50,000
			1/13/20	55,000
			2/21/20	55,000
			3/8/20	55,000
			3/22/20	55,000
16	W. S.		1/23/20	45,000
17	H. F. R.		1/21/20	50,000

will be seen that normal serum, 1 to 200 in the mixture, protected guinea-pig cells from hemolysis by sodium oleate up to strengths of from 1 to 55,000 to 1 to 40,000 and that with normal serum hemolysis was never seen with dilutions of sodium oleate greater than 1 to 55,000. The same was true of a wide range of other diseases as presented in Table IV.

There were only a few among the sera of cases with different types of anemia tabulated in Tables V and VI that did not have a lowered protective power against hemolysis as evidenced by the fact that hemolysis occurred in dilutions greater than

1 to 55,000. If the anemias not Addisonian in type are considered separately, Table V, it will be seen that nine of the twenty-one cases showed hemolysis in dilutions of 1 to 75,000 or over. Five of these showed hemolysis in dilutions greater than 1 to 75,000 as follows: three in 1 to 80,000, one in 1 to 85,000, and one in 1 to 90,000.

To be compared with the results of titrations on sera of cases in Table V are those on sera of pernicious anemia patients appearing in Table VI. Among the eighteen cases of pernicious anemia so studied the serum of thirteen showed hemolysis at 1 to 75,000 or above. In nine of the thirteen cases the protective power of the serum was lowered sufficiently to permit hemolysis in dilutions 1 to 85,000 or above. With the exception of one case, the serum taken after splenectomy from a patient with hemolytic icterus, the only sera which permitted hemolysis in dilutions greater than 1 to 85,000 were those of pernicious anemia patients, and among the eighteen titrated there were five that went beyond this point. The serum of one case of pernicious anemia did not inhibit hemolysis of the guinea-pig corpuscles in sodium oleate 1 to 105,000.

The five cases among those appearing in Table V, cases of anemia not Addisonian in type, that showed hemolysis in dilutions of over 1 to 75,000 merit special discussion. They were diagnosed (1) hemolytic icterus, (2) Banti's disease, (3) Hodgkin's disease, (4) cirrhosis of the liver, and (5) secondary anemia, cause undetermined.

1. The case of hemolytic icterus in a white man, fifty years old, has been under observation for years. On this admission he complained principally of weakness. He was emaciated and his skin was a pale yellow color. The spleen was much enlarged and the liver moderately enlarged. He had hemorrhoids. The Wassermann reaction was negative. The urine showed albumin but no bile or blood. There was always a positive test for urobilin. His blood on the last admission showed an anemia with a color index greater than one, (R. B. C.—2,752,000, Hb.—55 per cent), moderate anisocytosis and poikilocytosis, normal total and differential white blood cell count (W. B. C.—9000) and normal platelets. The resistance of the red blood cells to hypotonic salt solution was found markedly decreased on repeated examinations. There were 8.4 per cent reticulated red blood cells. As the patient's condition improved, the degree of anemia became less but the color index remained high. In this case the color of the skin and the high color index suggest by analogy that the anemia was due to a hemolytic process and the constant finding

TABLE IV

RESULTS OF TITRATIONS OF SERUM OF PATIENTS SHOWING LITTLE OR NO ANEMIA FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SODIUM OLEATE

No.	Name	Age	Wassermann reaction	Date	W. B. C.	P. M. N. %	R. B. C.	Hb.%	Last tube showing hemolysis	Diagnosis	Remarks
1	J. M. McK.	64	Neg.	7-31-19	4,920	....	3,936,000	78	50,000	Arterio-sclerosis and hypertension, myocardial insufficiency. Auricular fibrillation. Bell's palsy.	Diagnosis, 1904, by Dr. Osler, aneurism thoracic aorta.
2	N. M.	22	Neg.	7-30-19	10,800	64.	4,792,000	85	50,000	Post-influenzal asthenia, chronic tonsillitis, dental caries.	
3	P. I.	37	Neg.	7-31-19	13,080	....	3,864,000	80	50,000	Acute arthritis of the knee, gonorrheal(?).	
4	J. G.	69	Neg.	7-30-19	8,200	66.2	4,960,000	90	55,000	Dorsal and lumbar spondylitis.	
5	C. H. L.	21	Neg.	7-11-19	18,500	82.	4,782,000	90	55,000	Pulmonary infection right base, acute, not tbc.	
6	C. L. W.	57	Neg.	7-11-19	7,960	....	4,632,000	83	55,000	Arterio-sclerosis and emphysema. Myocardial insufficiency.	
7	T. S.	31	Neg.	8- 9-19	7,600	....	4,996,000	100	50,000	Addison's syndrome, tbc. adrenal (?).	
8	W. C. B.	36	Pos.	8- 9-19	8,800	62.	4,300,000	85	50,000	Luetic aortitis and aortic insufficiency; tabes.	
9	C. J.	60	Neg.	8-12-19	8,000	....	4,480,000	70	55,000	Carcinoma of stomach.	
10	W. M.	30	Neg.	8-13-19	7,600	....	4,472,000	80	50,000	Chronic nephritis.	Very œdematous. Bl. pr. 220/160.
11	A. W.	48	Pos.	10-22-19	10,600	69.1	4,632,000	77	55,000	Syphilis, aneurism of aortic arch.	
12	M. E. S.	60	Neg.	10-17-19	6,100	68.4	4,750,000	90	55,000	Carcinoma at head of pancreas.	
13	B. F. M.	27	Neg.	10-18-19	5,640	58.	5,072,000	90	55,000	Diabetes mellitus.	
14	D. W.	29	Neg.	9-23-19	6,000	72.4	4,976,000	100	50,000	Visceroptosis.	
15	E. K. U.	50	Neg.	7-23-19	25,000	....	11,000,000	130	50,000	Polycythemia.	
16	T. McB.	21	Neg.	7-29-19	7,800	....	4,760,000	84	55,000	Chronic infectious arthritis, right knee.	
17	L. B.	71	Neg.	7-29-20	8,760	....	4,480,000	98	60,000	Diabetes mellitus; furunculosis, healing.	
18	S. O.	70	Neg.	1-23-20	12,450	72.	4,976,000	95	50,000	Carcinoma of liver.	
19	J. T.	49	Neg.	1-26-20	9,200	74.	5,344,000	90	55,000	Myocardial insufficiency, auricular fibrillation.	
20	F. P.	27	Neg.	1-26-20	.....	....	.....	..	50,000	Dementia præcox.	Some paranoid elements in case.
21	R. K.	58	Neg.	1-28-20	10,000	61.	5,064,000	95	50,000	Diabetes.	
22	M. E. P.	58	Neg.	2- 2-20	11,200	72.	3,216,000	85	40,000	Obstructive jaundice, intense. Appears to be carcinoma.	
23	I. S.	36	Neg.	1-21-20	6,560	83.	4,632,000	70	45,000	Hypertension, achylia, cord symptoms. Macrocytes in blood. Slightly increased fragility of cells.	Complete recovery.
24	A. P.	72	Neg.	1-28-20	8,000	84.	4,544,000	94	60,000	Diabetes.	

of urobilin in the urine bears further evidence of this condition.

2. The case diagnosed Banti's disease, like so many others with this diagnosis, is under some uncertainty as to the true condition present. The patient, a white man thirty-nine years old, had had hematemesis and blood in the stools at fairly long intervals over a period of four years. In all there were sixteen moderately severe hemorrhages. The patient had been feeling weak and was anemic during this time and had had altogether five transfusions. About the time of the first hemorrhage he had noticed a tumor in the left upper abdominal quadrant and this had increased in size gradually during the four years before coming under observation here. On admission he was undernourished and anemic, there was papillary atrophy of the tongue, and the spleen was greatly enlarged. The liver was not enlarged. The Wassermann reaction was negative. An x-ray examination of the gastro-intestinal tract was negative. The stool was normal. Urine examinations were negative except that urobilin was found present on two occasions. The blood on admission showed a moderately severe anemia with a low color index, (R. B. C. 3,024,000, Hb. 35 per cent), marked poikilocytosis and moderate anisocytosis, slight diffuse basophilia, a severe leucopenia, (W. B. C. 3400), and moderate diminution of platelets. The resistance of the red blood cells to hypotonic salt solution was normal and there were 1 per cent reticulated red blood cells. The Goodpasture fibrinolysis test for chronic hepatic insufficiency was negative.

3. The case diagnosed Hodgkin's disease had been under observation for five years. When first seen he had had swellings on the sides of his neck for some time. His spleen was palpable. During the last five years the patient had been treated extensively with radium and x-ray so that his glands had never become very large but his weakness and anemia had gradually increased. On this admission the patient complained of weakness, shortness of breath, and cough. He was found to be extremely emaciated and his skin a lemon yellow color. There was a moderate general glandular enlargement. There were evidences of a mediastinal tumor, râles scattered throughout the chest, and some fluid on the right side. The liver and spleen were not enlarged. There were external hemorrhoids. The Wassermann test was negative. The stool examination was negative. X-ray examination of the mediastinum revealed a mass located there. Urine examination showed a slight trace of albumin. The blood showed a very severe anemia with a color index greater than one (R. B. C. 1,624,000, Hb. 38 per cent), moderate poikilocytosis and marked anisocytosis, a normal total white blood cell count (W. B. C.

TABLE V

RESULTS OF TITRATIONS OF SERUM OF PATIENTS SHOWING SOME ANEMIA NOT ADDISONIAN IN TYPE, FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SODIUM OLEATE

No.	Name	Age	Wassermann reaction	Date	W. B. C.	P. M. N. %	R. B. C.	Hb. %	Last tube showing hemolysis	Diagnosis	Remarks
1	J. G.	55	+	9-12-19	9,800	81	2,100,000	41	60,000	Carcinoma of stomach.	
2	W. L.	45	—	9-10-19	7,600	75	3,020,000	40	50,000	Secondary anemia. Hemorrhoids.	
3	C. R.	51	—	8-20-19	12,800	81	2,720,000	18	65,000	Abdominal carcinoma-cæcum. Intense secondary anemia.	
4	S. P.	31	—	9-24-19	6,200	..	2,506,000	62	45,000	Aestivo-autumnal malaria.	
5	McC. D.	19	—	9-25-19	4,200	83	3,920,000	65	60,000	Pleurisy with effusion. Tbc. of spine, sternum and tibia. Secondary anemia.	Anemia shows no evidence of regeneration.
6	I. L. D.	55	—	9-30-19	9,000	68	2,864,000	48	65,000	Myocardial insufficiency. Chr. nephritis. Goitre.	
7	E. H.	38	—	10- 6-19	5,500	..	2,600,000	38	45,000	Myeloid leukemia.	
8	W. R. D.	34	—	10- 8-19	3,400	67	3,024,000	35	80,000	Banti's disease. Splenomegaly.	
9	S. B.	47	—	11-25-19	19,200	82	2,960,000	72	65,000	Combined sclerosis.	
10	J. R.	70	—	1-14-20	8,600	85	3,600,000	40	60,000	Metastatic carcinoma of bones, glands, liver, lungs, etc.	
11	A. T. W.	50	—	1-28-20	6,720	50 2 myeloc.	3,352,000	60	70,000	Congenital hemolytic jaundice.	Splenectomy Feb. 20, 1920.
				3-15-20	9,000	..	2,752,000	55	65,000		
				4- 5-20	10,800	..	3,128,000	58	90,000		
12	B. R.	29	—	2- 2-20	5,600	80	2,616,000	55	65,000	Typhoid fever.	Patient extremely ill. Very pale.
				2- 4-20	3,640	..	3,184,000	52	75,000		
				2- 6-20	4,880	46.66	3,216,000	62	70,000		
				2-18-20	7,120	50	4,964,000	58	75,000	.....	Patient is a febrile now.
									50,000	.....	Three months after recovery.
13	A. W. F.	34	—	2- 6-20	3,600	65	2,620,000	40	65,000	Cirrhosis of liver. Cancer of liver (?)	Enlarged liver and spleen.
14	W. J. M.	50	—	2-16-20	23,760	..	1,896,000	28	60,000	Tumor of bladder. Secondary anemia.	Patient very ill.
15	J. R.	39	—	2-16-20	3,340	64	1,992,000	29	70,000		
				2-18-20	5,320	61	1,880,000	27	80,000		
16	D. T.	47	....	2-23-20	21,560	..	.....	37	75,000	Myomata uteri (submucous) necrotic.	
17	M. E. J.	48	—	2-16-20	7,080	67	3,796,000	65	65,000	Cirrhosis of liver?	
				2-18-20	.....	..	.....	..	80,000		
				2-21-20	8,840	67	4,864,000	90	80,000		
18	A. W. V.	52	—	2-27-20	9,240	81	1,624,000	38	85,000	Late stage of Hodgkin's Disease.	Were there nothing else to explain the blood picture it might have been P. A. Cl > 1.
				3- 8-20	5,000	..	2,456,000	42	85,000		
19	J. G.	..	....	1-19-20	840	..	1,200,000	20	75,000	Primary aplastic anemia.	
20	E. J.	20	Doubtful.	3-29-20	17,200	77	1,552,000	22	75,000		
21	S. C	23	—	10-14-19	6,200	..	3,800,000	76	65,000	Typhoid fever.	

9,240) with a slightly increased percentage of polymorphonuclear neutrophiles, and a moderately decreased number of platelets. The resistance of the red blood cells to hypotonic salt solution was normal. There were 2.2 per cent reticulated red blood cells. As the anemia improved, the color index came down to a little below one but was always high. The blood picture of this patient resembled that of pernicious anemia very much. It differed from it in that there was a higher total white count and percentage of polymorphonuclear neutrophiles than is usually seen in Addisonian anemia and the degree of poikilocytosis was less than is to be expected in an anemia so severe.

4. The case diagnosed cirrhosis of the liver was that of a woman forty-eight years old who had been ill for one year. She had been having abdominal pain, nausea and occasional vomiting, and acid eructations after meals. Her abdomen began to swell about one year before admission here and during this period she had been tapped four times. Recently her ankles had become swollen, she had had some shortness of breath on exertion and had suffered from intense thirst. She had noticed that her urine was scanty. When examined she was found to have fluid in her abdomen and œdema of the lower extremities. The spleen was not palpated but was found enlarged on percussion. The liver could not be felt. There were external hemorrhoids and dilated veins over the abdomen. The Wassermann test was negative. The stool was normal. The phenolsulphonephthalein excretion was normal. A test-meal showed nothing pathological. The urine was normal except that on one occasion sugar was found to be present. The blood was normal except for a moderate degree of anemia with a color index slightly less than one. (R. B. C 3,796,000, Hb. 65 per cent, W. B. C. 7,080).

5. The case diagnosed secondary anemia was that of a man thirty-nine years old who had received a very severe blow on the right side of the body about one year before. He had had pain and shortness of breath at intervals since. On rare occasions he had spat blood. He had been unable to work since the injury. During the last few months he also had had some pain after eating, and sometimes three or four watery stools a day. When examined he was slightly dyspnœic, was emphysematous, and very pale. The Wassermann test was negative. An x-ray examination of the gastrointestinal tract was negative. Examination of a test-meal was negative. The stool showed nothing pathological, notably no blood. The urine was negative. The blood showed a severe anemia with a color index greater than one, (R. B. C. 1, 540,000, Hb. 32 per cent, W. B. C. 7,070). As the anemia improved, the

TABLE VI

RESULTS OF THE TITRATIONS OF SERUM OF PATIENTS WITH ADDISONIAN ANEMIA FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SODIUM OLEATE

No.	Name	Age	Wassermann reaction	Date	W. B. C.	P. M. N. %	R. B. C.	Hb. %	Last tube showing hemolysis	Diagnosis	Remarks
1	H. H. C.	66	—	8- 6-19	2,600	58	670,000	15	80,000	Pernicious anemia.	Hemolysis probably higher but tubes not run further.
				9- 2-19	4,400	40	1,900,000	24	75,000	.....	Has had three transfusions since last test.
				10- 4-19	5,300	60	3,224,000	56	90,000	.....	Patient very much improved. Discharged.
2	E. B.	43	—	10-16-19	9,800	..	1,370,000	30	95,000	Pernicious anemia.	
				10-27-19	7,000	..	1,720,000	38	80,000	.....	Transfusion five days ago. Since then patient feels much better.
				4- 5-20	1,950	45	1,768,000	22	85,000	.....	Transfusion March 18.
3	J. G. P.	33	—	8- 8-19	9,000	..	2,152,000	68	85,000	Pernicious anemia.	
4	J. V. P.	56	—	8-14-19	2,920	50	1,688,000	50	70,000	Pernicious anemia.	
5	J. A. H.	41	—	9- 5-19	8,600	72	1,500,000	38	75,000	Pernicious anemia.	Patient not very ill.
6	S. C.	66	—	10-20-19	8,760	..	1,624,000	29	95,000	Pernicious anemia.	
				10-31-19	4,880	..	2,388,000	35	90,000	.....	Patient had transfusion 3 days ago. Feeling better. Died Jan., 1920.
7	R.	..	....	10-11-19	.....	..	510,000	9	105,000	Pernicious anemia.	Patient died 12 hours after serum was taken.
8	M. G.	44	—	12-11-19	3,500	53.5	1,650,000	36	75,000	Pernicious anemia.	
9	T. E. C.	59	—	2-27-20	6,200	66	1,524,000	35	70,000	Pernicious anemia. Mitral stenosis.	Not certain patient does not have an active endocarditis.
				3- 5-20	4,600	56	1,584,000	34	90,000	.....	Active endocarditis seems very questionable.
10	D. W.	35	—	3- 5-20	4,800	..	1,470,000	43	85,000	Pernicious anemia.	Patient not very ill.
11	A. M.	30	—	3- 5-20	10,000	55	1,770,000	45	85,000	Pernicious anemia.	Patient not very ill.
12	H. L. McW.	31	—	3- 5-20	2,440	33	824,000	28	65,000	Pernicious anemia.	
				2-15-20	1,520	32	1,544,000	39	70,000	.....	Transfusion (600 c. c.) 3 days ago.
				2-23-20	2,440	34	1,632,000	40	70,000		
13	F. T. H.	35	--	1-16-20	4,400	67	736,000	14	85,000	Pernicious anemia.	
14	F. L.	35	—	3-31-20	2,700	48	1,250,000	30	65,000	Pernicious anemia.	
15	S. F. M.	47	—	3-22-00	5,960	67	1,265,000	27	60,000	Pernicious anemia.	
				3,29-20	5,680	65	1,198,000	27	70,000		
16	B.	40	....	3-29-20	4,400	62	1,230,000	35	75,000	Pernicious anemia. Amœbic dysentery.	
17	S. E.	53	—	1-21-20	5,310	64	2,236,000	46	55,000	Pernicious anemia.	Patient has had no transfusion.
18	R. E. W.	45	—	5-10-20	2,350	68	2,064,000	35	80,000	Pernicious anemia.	

color index became a little less than one but always remained high.

These cases have been presented in some detail because the anemia present was associated with other findings of special interest. It was not in any one of these five cases a frank secondary anemia as that due to the blood loss of hemorrhage or to myelopoietic hypofunction of infections or cachexias: nor was it the type of anemia due to disturbances of iron metabolism as in chlorosis and some anemias of children. The case of hemolytic icterus was a typical case, showed all the accepted evidences of an anemia due to blood destruction in the body, and may be considered a hemolytic anemia. Three of the four remaining cases had high color indices, two of them greater than one. The remaining case that had a low color index showed urobilin in the urine. The most interesting feature, however, is that in four of the five cases there were evidences of involvement of the spleen in the disease process. Except for hemolytic icterus, the exact status of those anemias associated with disorders of the spleen as in these cases has not been established; and the exact mechanism of the anemia seen in diseases involving the spleen has not yet been explained.[16] In this connection it is interesting to note that with one exception the only cases tabulated in Table V (cases of anemia not Addisonian in type) that showed marked diminution of protective power of the serum against hemolysis could be assigned to this group of cases without any hesitation. The type of anemia in the fifth case diagnosed secondary anemia, cause undetermined, with a color index greater than one, is difficult to determine.

TABLE VII

A. Tabulation of the Number of Cases According to the Highest Dilutions of Sodium Oleate Showing Hemolysis of Guinea-Pig Corpuscles in the Presence of the Respective Sera

(1) Normal persons and patients showing no anemia—41 cases.
(2) Cases of Addisonian anemia—18 cases.
(3) Cases of other types of anemia—21 cases.

Dilutions of sodium oleate	45,000	50,000	55,000	60,000	65,000	70,000	75,000	80,000	85,000	90,000	95,000	100,000	105,000	Total
Cases with no anemia..	6	20	13	2	..	..	..	..	..	..	..	..	..	41
Cases of Addisonian anemia	..	..	1	..	1	3	3	1	4	2	2	..	1	18
Cases with other anemias	2	1	..	4	5	..	4	3	1	1	..	..	..	21

B. Tabulation the Same as Above Except That the Cases Showing Evidences of Involvement of the Spleen are Included with the Cases of Addisonian Anemia

Dilutions of sodium oleate.	45,000	50,000	55,000	60,000	65,000	70,000	75,000	80,000	85,000	90,000	95,000	100,000	105,000	Total
Cases with no anemia..	6	20	13	2	..	..	..	..	..	..	..	..	..	41
Cases of hemolytic anemia and splenomegaly	..	..	1	..	1	3	3	4	5	3	2	..	1	23
Cases with other anemias	2	1	..	4	5	..	4	..	..	..	..	..	..	16

To sum up the findings, among the sixty-two sera of normal people and of patients suffering from a wide range of diseases without anemia or with anemia not Addisonian in type presented in Tables III, IV, and V, there were only nine that showed hemolysis in dilutions of sodium oleate greater than 1 to 65,000, and five of these have been specially discussed. Among the eighteen sera from cases of Addisonian anemia appearing in Table VI all but two showed hemolysis in dilutions of sodium oleate greater than 1 to 65,000, many of them in very much greater dilutions. These results appear in summary in Table VIIA. If those cases which are not pernicious anemia but are associated with conditions in which the spleen is involved in the disease process are included with the pernicious anemia cases, the results are very striking (Table VIIB).

TABLE VIII

A Comparison of the Protective Power of the Sera of All Cases of Addisonian Anemia with R. B. C. Counts Over Two Million with That of the More Severe Secondary Anemias Tested

PERNICIOUS ANEMIA

Name	R. B. C.	Hb. %	Highest dilution of sodium oleate showing hemolysis
H. H. C.....	3,224,000	56	90,000
J. G. P.....	2,152,000	68	80,000+
S. C.....	2,388,000	35	90,000
S. E.....	2,236,000	46	55,000
R. E. W....	2,064,000	35	80,000

SECONDARY ANEMIA

Name	R. B. C.	Hb. %	Highest dilution of sodium oleate showing hemolysis	Diagnosis
C. R.....	2,720,000	18	65,000	Carcinoma of cæcum.
J. G.....	2,100,000	41	60,000	Carcinoma of stomach.
S. P.....	2,560,000	..	45,000	Estivo-autumal malaria.
E. H.....	2,600,000	38	45,000	Myeloid leukemia.
W. J. M..	1,896,000	38	60,000	Tumor of bladder.
G. R.....	1,992,000	29	70,000	Severe secondary anemia cause undetermined.
S. R.....	2,700,000	47	60,000	Metastatic carcinoma of bone.
G.....	1,200,000	20	75,000	Primary aplastic anemia.

That the amount of protective power exhibited by the serum was not dependent solely upon the degree of anemia, as contrasted with the type, is shown in Table VIII. Here all the cases of pernicious anemia with more than 2,000 000 red blood cells per cubic millimeter are compared with the more severe secondary anemias.

In Table IX the results of repeated titrations on one case of pernicious anemia are tabulated. From this it will be seen that the protective power of the serum fluctuated markedly, this to be compared with the relative stability of protective power of normal serum as exhibited by a normal

control, (Case 15, Table III). These fluctuations in the protective power of the serum in the case of pernicious anemia so studied were independent of changes in the blood picture. It was noteworthy, however, that when the titration of the protective power of the serum showed a value approaching normal, the patient felt better, and when the protective power was low, he seemed to be more ill. This is in line with the observations of all clinicians who have followed these cases closely, that the feeling of well-being of the patient or the reverse seems to be independent of his blood picture at the time. These fluctuations, apparently dependent upon the clinical

TABLE IX

THE RESULTS OF REPEATED TITRATIONS OF THE SERUM FROM A PATIENT WITH ADDISONIAN ANEMIA FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SODIUM OLEATE

F. T. H. 35 years old. *Pernicious anemia.* Wassermann negative.

Date	W. B. C.	P. M. N. %	R. B. C.	Hb. %	Last tube showing hemolysis	Remarks
1/16/20	4,400	67	736,000	14	85,000	Transfusion 1/16/20 300 c. c.
1/19/20	3,400	..	848,000	20	70,000	Transfusion 1/17/20 300 c. c.
1/26/20	5,600	..	1,696,000	42	60,000	Transfusion 1/24/20 600 c. c.
2/2/20	10,800	70	1,880,000	43	50,000	
2/9/20	5,200	..	2,096,000	41	70,000	
2/16/20	3,400	57	1,872,000	44	70,000	Transfusion 2/13/20 600 c. c.
2/23/20	.....	..	.....	..	55,000	
3/1/20	3,600	64	2,832,000	56	75,000	Transfusion 2/26/20 600 c. c.
3/8/20	4,800	60	2,280,000	56	75,000	
3/15/20	4,600	71	2,285,000	55	60,000	
3/22/20	3,960	54	1,928,000	42	75,000	
3/29/20	3,080	45	1,690,000	24	80,000	Transfusion 3/29/20.
4/5/20	2,600	47	2,372,000	45	90,000	
4/27/20	3,000	49	1,272,000	27	100,000	
5/3/20	3,760	54	1,076,000	22	80,000	
5/10/20	1,960	35	536,000	13	90,000	
5/17/20	2,280	58	936,000	20	85,000	Transfusion 5/12/20.

condition of the patient, may explain the good titration values obtained for the sera of a few pernicious anemia patients titrated only once. This is borne out by the fact that of the four cases, Nos. 4, 12, 14 and 17, which showed sera with protective power very close to normal, three went into remission promptly, two of them without transfusions. Subsequent titrations on the one case that did not improve promptly, showed a diminshed protective power in spite of the fact that he had received transfusions. The fact that improvement in the titration value runs more or less parallel with the way the patient feels and precedes the improvement in the blood picture strongly suggests that many of the general symptoms of the patient with pernicious anemia, if not directly due to the lowering of the protective power of the serum itself, may depend upon the same underlying cause rather than upon the degree of the anemia alone. This is tantamount to saying what has been apparent to clinicians for some time, that in pernicious anemia the anemia is but one manifestation of a complex disease.

When this work was undertaken it was hoped that if any diminution in protective power of serum against hemolysis could be demonstrated in anemic conditions, it might prove to be a finding confined to Addisonian anemia and thus be of value as a diagnostic procedure in difficult cases. The five cases not pernicious anemias which showed hemolysis in the higher dilutions, and the several cases of pernicious anemia that do not show greater diminution in protective power than anemias of other kinds, make this method of study of little value in diagnosis. One explanation of the high protective values obtained in some of the pernicious anemia cases has been suggested; namely, the patients were in good condition when tested. The possibility should also be kept in mind, however, that the cases at present diagnosed "pernicious anemia" may include more than one disease and that this method of study may have differentiated them. Additional study is needed on this point. It is hoped that further study of the protective power of serum in various diseases, especially pernicious anemia, may show that the information afforded by this procedure is of value in prognosis, and will aid in determining when a transfusion is indicated. Transfusion may improve the protective power of the serum slightly or markedly, but always results in some improvement. Sometimes this improvement, as judged by the titration of the protective power of the serum, does not last; sometimes it becomes progressive.

CONCLUSIONS

From the results of tests made of the protective power of human serum against hemolysis of guinea-pig cells by sodium oleate certain conclusions may be drawn.

(1) The protective power of the serum of normal persons and of patients suffering from a wide range of diseases without anemia is remarkably constant in degree.

(2) In anemias of various types the protective power of the serum is diminished.

(3) This diminution is most marked in anemias which are hemolytic in character and in conditions in which the spleen is involved in the disease process.

(4) In pernicious anemia, the diminution of protective power of the serum is very striking, both in degree and the regularity with which it is found.

(5) The protective power of the serum in cases of pernicious anemia varies greatly, but seems to parallel more closely the general condition of the patient than the blood picture at the time the titration is done.

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THE COAGULATION TIME OF CITRATED PLASMA ON RECALCINATION

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The determination of the coagulation time of the blood is of great importance in the hæmorrhagic diathesis, and more especially—as Sahli's fundamental work has shown—for the diagnosis of hæmophilia, which in sporadic cases may be somewhat difficult.

Having tried both the capillary methods (according to Wright's modification) and those based on an examination of larger amounts of venous blood, I have become convinced that these give very unreliable results.

The technique advised by Howell in 1914 may, with some alterations, give better results. The method consists in observing the coagulation time of optimally recalcinated oxalated plasma and is carried out as follows:

Four cubic centimeters of venous blood are mixed with 0.5 c. cm. of 1 per cent sodium oxalate and shaken. After centrifugalization, 5 drops of the plasma are dropped into each of four small test-tubes; one then adds, respectively, 1, 2, 3 and 4 drops of .5 per cent calcium chloride to the tubes and rotates the stand 90° every minute till the liquid in one of the tubes stiffens.

Howell's determinations were carried out at room temperature and showed a coagulation time (prothrombin time) of about 10 minutes, a marked protraction being found only in hemophilia.

My object now has been to modify this procedure so that it might be carried out on citrated blood on the same specimen as used for counting the platelets by Oluf Thomsen's method.*

The method is open to criticism for various reasons:

1. Most authors agree that the temperature has a decided influence on the coagulation time, whereas Howell uses room temperature, a very variable factor.

2. The conditions of the experiment are different from those naturally present in shed blood; consequently, the results cannot be compared.

3. The coagulation time depends upon the platelet content of the plasma which varies with the intensity of the centrifugalization.

In the elaboration of the method of the following experiments I have tried to overcome these objections. My technique in brief is as follows:

An *unsilvered* half-liter Dewar (vacuum) flask is provided with a cork with five holes, one for a thermometer and four for the miniature test-tubes. Before the experiment the flask is filled with water at 37° C., which will then remain at a temperature between 37-33° C. for several hours.

About 4.5 c. cm. of blood are taken with a curved needle into a centrifuge tube divided into tenths of a c. cm. and containing .5 c. cm. of 10 per cent sodium citrate solution. after starting the stop-watch, 2, 3, 4 and 5 drops of 1 per cent sedimented—excepting in cases of polycythæmia—so as to allow one to draw off .1 c. cm. of plasma for each of the four miniature test-tubes which have previously been inserted into the holes in the cork of the Dewar flask.

The test-tubes must be kept scrupulously clean and must have an internal diameter of 9-10 mm. The right size may be selected with a Hegar dilator or some similar instrument.

One then adds, going from left to right, 8, 7, 6 and 5 drops, respectively, of physiological saline solution to the tubes, and, after starting the stop-watch, 2, 3, 4 and 5 drops of 1 per cent crystallized calcium chloride solution ($Cacl_2, 6H_2O$). Pipettes giving 20 drops to the cubic centimeter were employed.

Every thirtieth second the flask is then rotated to the horizontal position until at last the surface of the liquid in one of the tubes (generally the second or third) fails to move. The interval between the recalcination and the solid clotting of the optimally recalcinated glass is called the coagulation time, though it marks neither the beginning of the coagulation, which may be observed half a minute earlier, nor the

* Gram: Arch. Int. Med., Chicago, 1920, XXV, 325.

end. In normal cases, that is, in persons not suffering from hæmophilia or platelet deficiency, the coagulation time is found to lie between 3 and 6 minutes inclusive.

The error on the determination is relatively slight since double specimens will show identical values or variations of one-half or rarely 1 minute and always within the said boundaries.

TABLE 1

DOUBLE SPECIMENS

Name	Diagnosis	c.-t. 1 min.	c.-t. 2 min.
H. W.	Ulc. ventr.	4½	4
A. P.	Colitis	3½	3½
A. H.	Ulc. ventr.	4	4
N. S.	Diabetes	4½	4½
H. W.	Ulc. ventr.	3½	3½
K. P.	Vertigo	4	4
C. J.	Normal	4½	4½
J. A.	Ischias	5	5
S. C.	Normal	3½	3½
N. C.	Normal	4	4½
A. J.	Normal	5½	4½

The time elapsing after the taking of the blood is—within 5 hours—of no consequence, as shown in Table 2.

TABLE 2

OBSERVATIONS ON THE SAME SPECIMEN AT VARYING TIMES AFTER THE TAKING OF THE BLOOD

Name	Diagnosis	1 hour c.-t. min.	3 hours c.-t. min.	5 hours c.-t. min.
A. J.	Obstipation	5½	5	..
K. K.	Pneumonia	5½	..	5½
A. H.	Ulcus ventr.	4	..	4
M. O.	Neurasthenia	5	4½	5
J. E.	Graves' disease	4½	4	4½
W. K.	Aplastic anæmia (platelet deficiency)	7½	7	..

The reasons for the technique selected are the following: Constant temperature is necessary (Addis and others). This is shown definitely by the experiments in Table 3, which gives the result in five cases at varying temperatures, larger blood specimens (20 c. cm.) being used to procure sufficient plasma. The advantage of observing the coagulation at 35° C. is obvious, since the variations in coagulation time due to a given change of temperature increase rapidly as the temperature becomes lower.

TABLE 3

INFLUENCE OF TEMPERATURE ON COAGULATION

Diagnosis	15° c.-t. min.	20° c.-t. min.	25° c.-t. min.	30° c.-t. min.	35° c.-t. min.	40° c. c.-.t min.
Normal	15	9	4½	3½	3½	3½
Normal	15½	9	6½	5	4½	4½
Cardialgia	16½	11	5	4	4	3½
Ulc. ventr.	13½	9	5½	4	3½	3½
Neurasthenia	14½	9	6	4	4	4½
Mean values	15	9.4	5.5	4.1	3.9	3.9

The use of the unsilvered vacuum flask ensures a constant temperature for hours, and obviates the necessity of extracting and cooling the tubes for inspection every thirtieth second.

In laboratories possessing a thermostat room the determinations may conveniently be carried out in this, thus doing away with the flask. The test-tubes used must be clean and have a constant size, since the coagulation time is a product of the innate qualities of the blood and its surroundings during the clotting.

The recalcination used is the result of experience which showed that the optimal recalcination always took place in the tubes containing 2, 3, 4 and 5 drops of a 1 per cent $CaCl_2$, $6H_2O$. If instead of a 1 per cent a 3 per cent solution is used, the recalcination must be 1, 2, 3 and 4 drops. Cases of protracted coagulation time, where the optimal recalcination was 2 or 5 drops, were controlled afterwards with 1 or 6 drops, respectively, no errors being found.

The variations due to different recalcination are shown in Table 4.

TABLE 4

COAGULATION TIME VARIATIONS DUE TO DIFFERENT RECALCINATION

Name	Diagnosis	1	2	3	4	5	6	7	8	Drops
K. B.	Normal	10½	6	4½	4	5½	6½	8	7½	Min.
J. E.	Normal	7½	5½	4½	5	6½	..	..	..	Min.

It will be seen that too low recalcination is much more effective in lengthening the coagulation time. The addition of sodium chloride solution to make the volume uniform in all tubes seems not to be strictly necessary, though I have no experiments to prove it. The fibrinogen content does not influence the coagulation time, even very low values being sufficient to cause solid clotting. Determinations of the fibrin percentage have been carried out in all cases.

The method presented differs from the original one of Howell inasmuch as it uses the sedimentation plasma containing the platelets in stable suspension.

This may cause difficulties in healthy men with very high red cell counts; these, however, may be obviated by taking double specimens in such cases. The reason for doing this lies in the fundamental importance of platelets for the coagulation time.

By centrifugalizing for 1¼ hours with 3000 revolutions a minute, all platelets may be precipitated, leaving a clear plasma which generally coagulates in from 8 to 14 minutes at 35° C. However, the clotting point is never so definite in this plasma, though the results may be quite interesting as a supplement to the examination of sedimentation plasma.

Any intermediary values found with plasma centrifugalized at low speed or only for a short time cannot be used. The difference between plasma centrifugalized and not centrifugalized is shown in Table 5.

Although the coagulation time is doubled in normal specimens, when the platelets are completely thrown down, this is not the case in pernicious anæmia with protracted coagulation time due to platelet insufficiency, as will be shown later.

In order to see whether the variations in coagulation time observed in recalcinated citrated plasma corresponded with variations in the coagulation time of venous blood, I have

carried out a series of observations of the coagulation time of venous blood.

The results were not very satisfactory, inasmuch as double specimens varied greatly, all determinations in normals however lying between 3 and 8 minutes (60 cases). Through a curved needle 1 c. cm. of blood was run down into a broad, flat-bottomed glass tube kept immerged in the Dewar flask.

TABLE 5

Name	Diagnosis	Sedimentation platelets c.-t. min.	Centrifugalized platelets c.-t. min.
O. J.	Nephritis	5½	13
L. K.	Cancer ventr.	4½	11
M. J.	Polyarthritis	5	12
G. A.	Normal	4	8½
H. N.	Normal	4½	12½
M. J.	Normal	4	11
G. K.	Normal	5	9
I. P. G.	Normal	4	11
J. J.	Normal	5	12
D. S.	Normal	4½	10½
N. J.	Dystr. musc.	5	14
J. N.	U. V.	5	9
X. X.	Hæmorrh. corp. vitrei	5½	13
K. H.	Fb. rheum.	3½	11
A. F.	An. pern. (remission)	5½	9½
E. L.	An. pern. (remission)	4	10½
K. J.	An. pern. (remission)	6	14
M. J.	Fb. rheum.	6	10
A. K.	Influenza	6	10½
H. W.	Icterus chron. 1 yr.	5½	12
J. O.	Vulnus oculi	5	9
E. P.	Pulm. tub.	5	9½
O. J.	Obstipation	4½	11½
Mean values		5	10½

The flask is turned to the horizontal position every thirtieth second till the blood stiffens. The narrow test-tubes used for the coagulation of plasma could not be used on account of the viscosity of the blood, which was apt to obscure the results.

The Lee & Vincent *Ca-in-vitro* test was carried out in many cases without ever showing definite evidences of lack of calcium even in cases of rather severe icterus of long duration. Table 6 shows a comparison between the results of determinations of the coagulation time of blood and plasma in some pathological cases, one case of hæmophilia and four cases of pernicious anæmia. The platelet count by O. Thomsen's method is given on the extreme right.

TABLE 6

Name	Diagnosis	c.-t. of plasma normal, 3-6 min.	c.-t. of blood normal, 8-8	Platelet count normal, 200,000-550,000
P. H.	Hæmophilia	16	30½	402,000
A. P.	An. pernic.	8½	12	74,000
A. P.	An. pernic.	6½	9	83,000
J. N.	An. aplast. pern.	10	9	16,000
A. M. P.	An. pern.	11	10	7,000

The protracted coagulation time is evident both in blood and in plasma, the lengthening being most marked in the blood in hæmophilia, in the plasma in pernicious anæmia. The influence of temperature on the coagulation time of venous blood is the same as on plasma, as shown in Table 7.

TABLE 7

Name	Diagnosis	Blood c.-t. minutes 35° C.	Blood c.-t. minutes 18° C.
A. L.	Diabetes	6½–7½	12½
J. J.	Ischias	6½–7	11½

If we tabulate the plasma clotting-time determinations carried out in normal persons and patients with "indifferent" diseases with normal platelet counts, we find the following:

TABLE 8

NORMAL INDIVIDUALS

Coagulation time of plasma	Number of individuals	Number of determinations
3 minutes	4	4
3½ "	20	22
4 "	39	44
4½ "	32	38
5 "	38	45
5½ "	15	15
6 "	5	5
	109	173

In repeated determinations at weekly intervals in the same individual the variations are larger than in double specimens taken at the same time; the variations, however, are always within normal bounds.

Shortening of the coagulation time to 2 minutes has been observed in a few cases. No pathological significance can be attributed to these results, as, generally, they were not found on repeated examination. These variations may be due to bad venapuncture or unclean glasses. However, in convalescing pneumonias and anæmia due to malignant tumors, where the platelet count is high, rather low periods of coagulation time are often but not always met with. In a smaller number of normal individuals, the coagulation time varied between 3½ and 6 minutes.

The pathologically protracted coagulation times fall naturally into two groupings, those with and those without platelet deficiency.

A decidedly low platelet count, less than 200,000 per c. mm., is found in pernicious and aplastic anæmia, in certain infectious diseases, influenza-pneumonia, typhoid, etc., and in a few other conditions.

In a general way one may say that the coagulation time is prolonged beyond 6 minutes when the platelets fall below 200,000; the phenomenon however is constant only when the platelet count is less than 100,000 per c. mm.

There is, of course—as may be seen from the following tables—a relation between the fall of the platelet count and the protraction of the clotting-time, but this is not a mathematical relation which would enable us to calculate the other, when one of the factors is known.

The reason for this lies not in the experimental error which may of course play a rôle, but in the varying size of the platelets found in pathological conditions. The effect of this "anisothrombocytosis" is that the clotting power of a given

number of platelets varies in different cases, just as the oxygen capacity of a given number of red cells varies in micro- or megalo-cytosis.

If in dealing with the first group of cases we turn to the observations in pernicious and aplastic anæmia, we find the result of the first examination in 22 cases, 18 pernicious and 4 aplastic anæmias, in Table 9.

All cases were either fresh untreated cases or newly admitted relapses, excepting Cases 7 and 8, which were in different stages of a remission at the time of the first examination. In addition to the coagulation time and the platelet count the hæmoglobin values are noted, to give an idea of the severity of the disease.

TABLE 9

PERNICIOUS ANÆMIA

	Name	Clotting-time, minutes	Platelets pr. c. mm.	Hb. per cent
1	E. L.	6	55,000	43
2	K. T.	8	58,000	36
3	A. F.	8	73,000	34
4	V. N.	7	140,000	76
5	A. M. P.	12½	13,000	13
6	C. S.	7	93,000	40
7	M. L.	7½	246,000	45
8	H. O.	6½	188,000	79
9	C. G.	5	116,000	52
10	M. T.	6½	193,000	56
11	A. P.	8	66,000	about 30
12	M. J.	4½	56,000	31
13	J. F.	9½	13,000	21
14	G. B.	6½	36,000	16
15	M. P.	11½	16,000	15
16	R. H. M.	11	21,000	25
17	I. W.	11½	22,000	28
18	E. O.	13½	4,000	21
19	J. N.	9½	20,000	28
20	W. K.	7½	105,000	aplastic 47
21	K. P.	9½	34,000	14
22	P. B.	7	72,000	38

The only cases showing a serious disagreement between platelet count and clotting-time are Nos. 7 and 12. Attention is drawn to the fact that the coagulation time of uncentrifugalized plasma in pernicious anæmia never exceeds that of normal centrifugalized plasma. In Table 10 the difference between uncentrifugalized plasma is shown in a few cases of pernicious anæmia.

TABLE 10

Name	Clotting-time minutes not centrifugalized	Clotting-time minutes centrifugalized	Platelet count per c. mm.
A. P.	8½	16	37,000
J. F.	9½	11½	13,000
J. N.	10	13½	11,000
J. N.	11½	13	5,000
V. N.	7½	12½	38,000
C. S.	7	10	93,000
E. O.	13½	16	4,000
A. P.	11	14½	6,000
E. L.	8½	10½	14,000
P. B.	7	8	72,000

It is apparent that the clotting time is only slightly prolonged by centrifugalization in these cases, three of the cases however slightly exceeding the normal limits for centrifugalized plasma. This might point to an essential difference between the platelet deficit in pernicious anæmia and in purpura, where Hess has found normal plasma clotting-times which were not prolonged by centrifugalization.

The variations in coagulation time during remissions and relapses in several cases follow the variations in the amount of platelets and are shown in Table 11.

TABLE 11

REPEATED DETERMINATIONS IN PERNICIOUS ANÆMIA. THE NUMERALS FROM ABOVE IN EACH SQUARE SHOW DATE, PLATELET COUNT, CLOTTING-TIME AND HÆMOGLOBIN

	6-9	19-9	13-10	26-1-20	8-3-20	....	....	....	Remission and relapse.
	55,000	23,000	113,000	197,000	14,000	....	....	....	
	6	9½	5	6	8½	....	....	....	
	43	28	59	66	19	....	....	....	
K. T.	29-12	5-1-20	26-1	8-3-20	....	....	....	....	Remission.
	58,000	38,000	75,000	194,000	....	....	....	....	
	8	7½	6½	4½	....	....	....	....	
	36	31	49	76	....	....	....	....	
A. F.	3-12	22-12	5-1	8-3-20	....	....	....	....	Remission.
	73,000	58,000	344,000	362,000	....	....	....	....	
	8	7½	5½	4	....	....	....	....	
	34	25	43	76	....	....	....	....	
V. N.	15-2	15-3	5-1	19-1	8-3	....	....	....	First two examinations during remission, then relapse and new remission.
	140,000	181,000	77,000	45,000	106,000	....	....	....	
	7	6	6	7½	6½	....	....	....	
	76	?	48	47	65	....	....	....	
J. N.	22-12	24-12	5-1	1-2	....	....	....	....	Died.
	20,000	11,000	5,000	16,000	....	....	....	....	
	9½	10	11½	10	....	....	....	....	
	28	24	26	12	....	....	....	....	
W.K.	20-2	25-2	....	....	....	....	....	....	Died.
	105,000	40,000	....	....	....	....	....	....	
	7½	11½	....	....	....	....	....	....	
	47	35	....	....	....	....	....	....	
C. G.	31-5	7-6	14-6	21-6	28-6	7-7	....	....	Remission.
	116,000	108,000	90,000	173,000	177,000	263,000	....	....	
	5	6½	7½	5	6	6	..	....	
	52	50	56	63	73	82	....	....	
A. P.	6-3	11-3	21-3	1-4	24-4	14-5	14-6	1-7	Slight remission, then relapse and death.
	66,000	61,000	74,000	55,000	83,000	95,000	74,000	37,000	
	8	7½	8½	6½	6½	7	7	8½	
	about 30	?	?	?	46	47	49	32	
I. W.	27-10	7-11	28-11	5-12	....	....	....	....	Died.
	22,000	41,000	42,000	26,000	....	....	....	....	
	11½	9	6½	7	....	....	....	....	
	28	30	36	31	....	....	....	....	
A. P.	25-2	262	....	....	....	....	....	....	Died.
	13,000	7,000	....	....	....	....	....	....	
	12½	11	....	....	....	....	....	....	
	13	13	....	....	....	....	....	....	

In three cases of myeloid leukæmia with normal or increased platelet count the coagulation time was normal.

In two such cases with periods of low platelet count and symptoms of a hæmorrhagic diathesis, the clotting-time was protracted, when the count was at its lowest.

TABLE 12

MYELOID LEUKÆMIA WITH PLATELET DEFICIT. THE NUMBERS FROM ABOVE IN THE HORIZONTAL COLUMNS GIVE DATE, PLATELET COUNT, CLOTTING-TIME, HÆMOGLOBIN AND LEUCOCYTES

M. J.	24-2	5-3	12-3	Slight improvement.
	75,000	118,000	134,000	Discharged for private reasons.
	6½	5	5½	
	47	....	....	
	249,000	....	....	
E. E.	24-9	16-12	....	Increasing anæmia.
	405,000	90,000	....	Died.
	5½	7½	....	
	?	33	....	
	22,500	18,200	....	

In one case of lymphatic leukæmia with normal platelet count the clotting-time was normal.

In three other cases with low platelet counts, the clotting-time was protracted in the two that showed the lowest number.

TABLE 13

LYMPHATIC LEUKÆMIA WITH PLATELET DEFICIT

J. B.	29–3	7–4	22–4	21–6
	170,000	175,000	156,000	139,000
	5	5	5	4½
	?	?	78	66
	?	?	247,000	63,100
C. M.	26–5	....	....	....
	49,000	....	....	....
	6½	....	....	....
	51	....	....	....
	3,200	....	....	....
A. S.	21–2	....	....	....
	134,000	....	....	....
	7	....	....	....
	35	....	....	....
	120,000	....	....	....

On the whole a platelet deficit in leukæmia seems not to protract the coagulation time as much as does a similar change in pernicious anæmia.

In a few other cases a low platelet count and possibly a functional insufficiency of the amount present may also have played a part in prolonging the clotting-time.

TABLE 14

PROTRACTED CLOTTING WITH DECREASED OR LOW NORMAL PLATELET COUNTS IN VARIOUS DISEASES

Name	Diagnosis					
E. M.	Pneumonia.	Date	10–5	....	....	Died.
		Platelets	247,000	....	....	
		Clotting-time	6½	....	....	
C. C.	Purpura.	Date	....	....	....	Died.
	Anæmia.	Platelets	112,000	....	....	
	Endocarditis.	Clotting-time	7	....	....	
O. P.	Acromegaly. Anæmia.	Date	7–2	17–2	24–2	
		Platelets	233,000	248,000	200,000	
		Clotting-time	8	6	8½	
		Date	7–3	....	....	
		Platelets	205,000	....	....	
		Clotting-time	7½	....	....	
I. O.	Tumor lienis. Anæmia.	Date	13–1	28–1	....	
		Platelets	157,000	247,000	....	
		Clotting-time	8½	6	....	
M. K.	Influenza.	Date	20–1	....	....	
		Platelets	216,000	....	....	
		Clotting-time	6½	....	....	

In several other cases of influenza and influenza-pneumonia evamined the coagulation count was normal; the platelet count, however, never went below 100,000 per c. mm.

The other group is represented only by three cases of hæmophilia, all three with a typical family history.

TABLE 15

HÆMOPHILIA

Name				
P. H.	Date	23–4	27–5	Pronounced case with hæmorrhage and joint affections.
	Platelets	402,000	457,000	
	Coagulation time	16	14½	
I. C.	Date	27–5	....	Pronounced case with hæmorrhage and joint affections.
	Platelets	394,000	....	
	Coagulation time	13	....	
S.	Date	26–6	....	Light case in an elderly man.
	Platelets	528,000	....	
	Coagulation time	7½	....	

Unfortunately I have not determined the coagulation time of centrifugalized plasma in these cases, the excess of plasma being used for other purposes.

However, I observed that a larger amount of plasma than was recalcinated with the optimal amount of $CaCl_2$ solution took some 50 minutes to clot firmly, the end point being very difficult to fix, since the coagulation proceeded very slowly.

It seems that by centrifugalizing one accentuates the difference between normal and hæmophilic blood, whereas in pernicious anæmia quite the opposite is observed.

Attention is drawn to the fact that the coagulation time in the first case of hæmophilia is longer than that of normal platelet-free plasma.

SUMMARY

1. A method is described by which the clotting-time of citrated plasma may be determined at 35° C. in tubes placed in an unsilvered Dewar flask. The procedure is a modification of Howell's principle of optimal recalcination, and may be performed on the same blood specimen as that employed for the Oluf Thomsen method of counting the platelets.

2. In normal individuals the clotting-time of the optimally recalcinated tube lies between 3-6 minutes for uncentrifugalized plasma, and generally between 8-14 minutes for platelet-free plasma, that has been centrifugalized for 90 minutes at high speed.

3. The reasons for choosing the first are the following:

(a) No centrifuge is needed.

(b) The change from the liquid to the solid state is sharper.

(c) The result shows changes both in the platelet count and in the clotting power of the plasma proper.

4. The necessity for maintaining a constant temperature (best 35° C.) in coagulation experiments is shown.

5. It is shown that the variations in clotting-time of optimally recalcinated plasma correspond with those of venous blood.

6. It is shown that the coagulation time of uncentrifugalized plasma is longer than normal in the hæmorrhagic diathesis with platelet deficit—pernicious anæmia, leukæmia and various other diseases.

7. In hæmophilia the coagulation time is very much longer than normal, although a perfectly normal number of platelets is present.

8. The method is of importance for the diagnosis of hæmophilia if combined with a determination of the platelet count after Oluf Thomsen's method. It may also reveal the existence of a hæmorrhagic diathesis in the course of diseases of the blood. If hæmophilia can be excluded, a prolonged coagulation time means that there is a numeral, or partially functional, deficiency of platelets, though the converse need not be true.

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THE ORIGIN OF HÆMANGIECTASES

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INTRODUCTION

The origin and nature of angiomata of the liver have been the subject of prolonged discussion by many authors. Two general ideas have been proposed: one, that angiomata are new growths; the other, that they are merely capillary dilatations resulting from various causes. Among the advocates of the new-growth theory some believe that they are primarily connective-tissue tumors and others that they are vascular in origin. Virchow[1,2] advanced the former idea, proposing that the process begins with a proliferation of connective tissue and that finally the newly formed capillaries dilate to form a cavernous ectasia. This theory has been advocated from time to time by different observers (Rindfleisch,[3] Burckhard[4]), although an explanation of the cause for the capillary dilatation is lacking. Ribbert[5] proposed the theory that hæmangiomata are new growths arising from the proliferation of misplaced vascular rests, which in turn result from developmental abnormalities. MacCurdy[6] has recently suggested that angiomata and many endotheliomata arise from anlagen of undifferentiated cells having the triple capacity of forming blood cells, blood vessels and lawless tumor cells.

In contrast, Borst[7] concluded that most angiomata are not new growths, but result from a metamorphosis of blood vessels with capillary ectasia and secondary connective-tissue proliferation, and that only rarely are they vascular neoplasms. Schmieden[8] was of the opinion that the liver cavernomata are developmental anomalies, and that their final form is determined by secondary regressive changes in the liver cells. Hæmorrhage, chronic passive congestion, a "hyperæmia ex vacuo" secondary to atrophy of the liver cells, capillary dilatation and even congestion of bile, as well as many other theories, have been advanced to explain the origin of these structures (Sheffen,[9] Benecke,[10] *et al.*).

This paper is based on the study of diffuse hæmangiectases involving particularly the liver, kidneys, and adrenal glands. The condition is interesting on account of its rarity. Furthermore, its study has suggested a mechanistic origin for angiomata. The theory is based upon fundamental principles of the development and growth of the vascular system and on certain observations which, although previously made, are a conspicuous feature of this case.

CASE REPORT

The case was one of general carcinomatosis from a primary tumor of Bartholin's gland. The clinical history pertains entirely to this condition. The abstract of the protocol presented below will include only those features which have a direct bearing on the angioma.

AUTOPSY No. 201.—*Liver.*—The liver weighs 3700 grams and measures 37 by 28 by 10 cm. Grossly, it shows a mottling of alternate dark red and lighter colored areas. On section the periportal areas are definitely outlined by dark red lines, and the lobules are distinctly seen.

Microscopically, the liver shows numerous blood channels situated chiefly in the periportal region, forming a chain of dilated sinuses surrounding the lobules (Fig. 1). In many areas this process extends beyond the confines of the peripheral zone, and the lobular architecture is wholly or partly obliterated by groups of dilated blood spaces that are typically cavernous in type. These sinuses are filled with blood, but tne effects of pressure on the surrounding parenchyma are not conspicuous. In other areas small dilated capillaries extend into the middle and central zones of the lobules, and in still other areas the picture of chronic passive congestion is seen. The blood channels are irregular in size and shape, intercommunicate freely, and are completely or partially separated from one another by septa of normal liver cells or bands of connective tissue. They are lined with a single layer of conspicuous endothelial cells. Actual formation of new capillaries is not seen in spite of a careful study by serial sections.

Perhaps the most interesting feature of the liver angioma is the evidence of hæmatopoiesis. The blood channels contain many immature cells of both the erythroblastic and myeloblastic series (Fig. 2).

Kidneys.—The two kidneys are similar. They are not enlarged, and externally, as well as on section, their appearance is normal except for congestion.

Microscopically, there is a less extensive, but only a slightly less conspicuous, hæmangiectatic process than was described for the liver. There is a diffuse involvement of the vessels of the glomeruli, labyrinth, and pyramids. All gradations are encountered, from slight dilatation of the vessels of a glomerulus to a practically complete involvement and conversion of the structure into a dilated vascular channel (Fig. 3). The other vessels show a dilatation of the telangiectatic rather than of the cavernous type. Although not a conspicuous feature, a few of the dilated blood spaces in the glomeruli show nucleated red blood cells as evidence of blood formation.

Adrenals.—The adrenals show a diffuse telangiectasis. The process involves the cortex extensively and the medulla to a lesser degree. There is definite evidence of blood formation in the presence of nucleated red blood cells and young cells of the myeloid series.

The heart muscle, the spleen, and pancreas also contain dilated vessels, but the process is not sufficiently definite to allow the conclusion that the same changes are present in these organs as were found in the liver, kidneys and adrenal glands.

In summary, there is extensive diffuse hæmangiectasis of the liver situated in the periportal region, an unusual type of bilateral diffuse hæmangiectasis of the kidneys involving all portions of the medulla and cortex, but most markedly the glomerular tufts, and a similar diffuse process in the adrenal glands. Furthermore, in all three organs evidence of hæmatopoiesis is easily recognizable.

* The author wishes to express his appreciation to Dr. J. T. MacCurdy for the use of his unpublished manuscript, "The Relation of Autochthonous Blood Cells and Endothelial Proliferation to Angiomata and Endotheliomata."

Discussion

The usual cavernomata of the liver are without clinical symptoms and are of theoretical interest only. They are relatively common at the autopsy table and their etiology has been widely discussed. They are frequently multiple, usually small, and sometimes are associated with angiomata of other organs. They may be on the surface or buried deep within the liver substance as sharply outlined, deep purplish-red areas. Microscopically, there is a variable amount of connective-tissue proliferation which sharply outlines the angiomata. They consist of numerous freely anastomosing, dilated, vascular channels, separated by thin septa of fibrous tissue or of liver cells.

The discussion of the relation of the blood channels of cavernomata to the normal vessels of the liver shows great difference of opinion. Many observers have made injections through the portal and hepatic veins, the hepatic artery and the cavernoma itself. Some have succeeded and others have failed to demonstrate a connection between the cavernoma and the general circulation. The majority of authors have not found a free connection, although confirmation of either view is easily found. In the face of such variable results, it is probable that the connection is not free in many cases.

There is also a great difference of opinion concerning the presence of a true growth of capillaries. MacCurdy [6] studied a series of thirty hæmangiomata involving the liver, spleen, intestine, skin, heart valves, kidneys, nasal mucosa, and the popliteal space, and states that: (1) all the cavernomata contain areas showing the simpler capillary type, (2) if numerous sections are made, growing capillaries are always found, some of which have endothelium proliferating to form several layers, (3) there are always areas showing proliferation of endothelium into the surrounding connective tissue and parenchyma, (4) evidence of blood formation is seen in all cases. The majority of cases on record do not confirm MacCurdy's view that true vascular growth is universally present.

On the other hand, his conclusion concerning the persistence of hæmatopoiesis has been confirmed by the author in recent studies of angiomata of the liver, skin, and testicle.* This observation has been made before. For example, Pilliet [11] describes six hæmangiomata and Schmieden [8] two showing evidence of blood formation. In addition to similar findings, in every case of his series, MacCurdy believes that he has found evidence of hæmatopoiesis in the literature, in the drawings and descriptions of other authors who have not appreciated the significance of the cells they described.

Circumscribed liver cavernomata are quite common in the literature, but a diffuse process such as is reported in this paper is relatively uncommon. Major and Black [12] report a case in which the liver weighed 18,160 grams and measured 35 by 44 by 11 cm. The liver tissue was largely replaced by a spongy network filled with clotted blood. The microscopic picture was that of large dilated blood channels. There was extensive destruction of the liver, and in some places an overgrowth of fibrous tissue with evidence of active connective tissue and capillary proliferation. The relation to the portal or hepatic veins was not discussed. In addition there was an hæmangioma of the skull and bilateral cystic adrenals. Mantle [13] describes a case in which the tumor measured 30 by 16 by 4 cm. Grossly, the right lobe was involved in a large cavernous angioma, while the left lobe was unaltered. Microscopically, the main mass of the growth showed irregular vascular channels separated by septa of dense fibrous tissue. The blood spaces were very large and were crossed by trabeculæ of fibrous tissue containing bile-ducts and occasional collections of hepatic cells. At the margin of the growth there was no capsule separating the tumor from the adjacent liver tissue and the vascular channels were dilated in the periportal zones of the neighboring lobules. In the left lobe there was also some dilatation of the branches of the portal canals but the liver substance was normal. Mantle [13] says, "This growth had apparently originated in the dilatation of pre-existing vessels rather than in a new formation. This is shown by the presence of hepatic cells and bile-ducts throughout the growth and by dilatation of the vessels of the left lobe." Schrohe [14] adds an example of an hæmangioma of the liver identical with the one contributed in this communication, except that evidence of hæmatopoiesis is not mentioned. Langer [15] extirpated a large angioma of the liver weighing 5000 grams and measuring 21 by 21 by 11 cm. The main mass was a typical cavernoma, but he describes a dilatation of the vessels in the normal liver substance at the periphery of the tumor, and, according to his illustrations, the dilatation was limited to the periportal area.

Borst [7] describes a case showing dilatation and enlargement around the central veins and says that interesting changes were encountered in different portions of the liver. These showed stretching of the vessels, then a telangiectasis, and finally, a cavernous metamorphosis. Roggenbau [16] reports a large hæmangioma involving the whole left lobe, the left half of the right lobe, and the quadrate lobe. The liver in this case measured 22 by 33 by 9 cm. The main mass showed typical irregular cavernous spaces. At the periphery of the various tumor masses, where the involvement was less marked, the central veins and their branches were dilated. Furthermore, there was a simultaneous proliferation of new vessels and connective tissue. M'Weeney [17] describes a case in which the liver weighed 5100 grams (180 oz.) and was transformed into a large angioma measuring 25 by 25 by 10 cm. There were also dilatation and widening of the branches of the central veins. Wagner [18] describes a similar case and MacCurdy [6] mentions one showing dilatation of the vessels in the central zones, but he does not consider this case analogous to the usual cavernoma.

There are also cases recorded of large angiomata of the liver removed at operation in which the condition of the remainder of the liver could not be determined.

The small number of cases of diffuse involvement of the liver fall into certain groups: one, in which there is a close relation

* This opinion was also confirmed in an hæmangioma of the skin of a new-born infant. The blood in the general circulation showed no evidence of haematopoiesis, but nucleated red blood cells were easily found in blood smears from the angioma.

to the portal vein and its branches; another, in which a similar relation to the central veins is described, and, as might be expected, some in which the extent of the process is greater and no such relation is apparent.

The usual cavernoma is circumscribed. It may be single or multiple and involve either the liver only or several organs. Diffuse hæmangiectases are rare and the association of such a diffuse process in the liver, kidneys, and adrenals as is found in the case reported here has not been previously described.

The Origin of Hæmangiectases

Before proposing an explanation for the origin of hæmangiomata, a review of some general principles of the development and growth of the vascular system is essential.

The first tissue of the embryo that exhibits unquestionable differentiation (Minot [19]) is the angioblast or primitive vascular anlage. The angioblast appears on the yolk-sac as a group of blood vessels forming the vitelline capillary plexus and covers the entire yolk-sac in the earliest human embryos. There have been two opinions held as to the further development: one, that there are other vascular anlagen derived from the mesoderm directly, and, accordingly, that there are many sources of early blood vessels; the other, that the entire vascular system is derived from growth and extension of the vitelline capillary plexus (Evans [20]).

For a long time it has been possible to show that all vessels formed subsequent to the development of the aorta arise as capillary sprouts from pre-formed endothelium. Bremer [21] has demonstrated in the head of a rabbit embryo of five somites that the aorta is represented by a capillary plexus coterminous with the vitelline capillary plexus. This fact probably indicates that the entire vascular system arises from a budding of the primary angioblast of the yolk-sac. The Swiss anatomist, Aeby,[22] expressed the opinion in 1868 that the vascular system originally existed as a uniform capillary meshwork of vessels. This idea has been strongly opposed until recently, when it has been shown by the injection of living embryos that the early vascular system does appear in a multiple capillary form. Evans [20] states that this represents the fundamental method of vascular growth, and that the larger vessels come into existence only by the enlargement of certain fortuitously situated capillaries which assume a larger and larger function and develop into arteries at the expense of other capillaries which accordingly must regress. Growth, however, is not a function confined to the capillary plexus alone, as branches may also arise from embryonic arteries after they are differentiated as such. The most familiar example of the operation of this principle is seen in the growth of granulation tissue which, in its beginning, is a highly vascular tissue consisting of numerous capillary loops, while the end result, scar tissue, is relatively avascular.

In some organs, the liver for example, the blood vessels arise in a different way (Mall [23]). Here, the method is invasion of large venous trunks, the omphalomesenteric veins, by the glandular tissue in such a way that the main trunk becomes broken into a large number of smaller vessels. The vessels formed in this way (the so-called sinusoids) are markedly irregular and are often much larger than the usual capillaries.

In his study of the structural unit of the liver, Mall [23] has discussed the histomechanical principles of Thoma [24] in relation to the vascular growth in that organ. Thoma's three laws are as follows:

"The increase in the size of the lumen of the vessel, or, what is the same thing, the increase in the surface of the vessel wall, depends on the rate of the blood-current. The surface of a vessel wall ceases to grow when the blood-current acquires a definite rate. The vessel increases in size when this rate is exceeded, becomes smaller when the blood-stream is slowed, and disappears when it is finally arrested.

"This law which brings the growth of the surface of the vessel wall into dependence upon the rate of the flow of blood is, I consider, the first and most important histo-mechanical principle which determines the state of the lumen of the vessel under physiological and pathological conditions.

"A second histo-mechanical principle may be added to this, *viz.*, the growth in thickness of the vessel wall is dependent upon its tension. Further, the tension of the wall is dependent upon the diameter of the lumen of the vessel and upon the blood pressure.

"The third histo-mechanical principle has not hitherto been so completely demonstrated as the first two. It will, therefore, be put forward merely as an hypothesis, which runs as follows: increase of blood pressure in capillary areas leads to new formation of capillaries."

In his study of the liver, Mall has shown that the first two principles of Thoma are applicable, but in reference to the third principle that factors other than blood pressure are of importance in the formation of new capillaries, we may quote Thoma [24] further:

"According to the generally accepted view of the problem of circulation, which was formerly quite sufficient to serve as a basis for the account of its general disturbances, the pressure, the rate, and the amount of blood-flow appeared to be directly dependent upon the action of the heart. According to the view given here, on the other hand, it is the metabolic processes in the organs, which determine first for the individual organs, then for the whole of the organs—that is, for the circulation as a whole—the amount of blood propelled within a given time, its pressure, and its rate of flow. In this case the working-power of the heart appears as the equivalent of the sum of the histo-mechanical demands made by the organs."

Mall [23] and Thoma [24] find that the capillaries of like component parts of an organ are of equal size and length, and that the rapidity of the circulation through them is equal. This is brought about by the action of mechanical laws; with a greater flow of blood, a capillary dilates and becomes converted into an artery or vein, other channels in which the rate of flow is constant remain as capillaries, and, lastly, some channels offering great resistance are slowly traversed and gradually disappear.

In his study of the liver, Mall [23] points out that the capillaries of the liver lobule consist of long capillaries and short capillaries. By an elaborate model of the lobule, he demonstrated that the long and short capillaries are equally favored by the circulating fluid. This was apparently a stumbling block. Further study showed, however, that the

long capillaries of contiguous lobules converge toward that portion of the interlobular fissure in which there are no collecting veins. This immediately suggested that some of the main capillaries would be converted into veins, which is the case in growing livers, but with each new vein a new vascular unit is formed and two new nodal points complicate the picture (Mall[25]).

From this very brief consideration of the embryology of the circulatory system in general, of Thoma's laws, and of their application to the liver, it is evident that: (1) the general mode of development of the vascular system is in the form of a primary capillary plexus; (2) certain capillaries undergo regression, others remain stationary, and others develop into larger vessels forming the arteries and veins to the part; (3) the growing capillary system in obedience to the laws of mechanics is a changing and variable structure.

On this basis, many vascular abnormalities are easily explained. A persistence of certain parts of the original capillary plexiform anlage may explain the origin of the hæmangiomata or hæmangiectases of various organs. The complicated, ever-changing vascular system of the liver is likely to be a favorite site for such changes. The explanation of why these capillaries persist may offer some difficulty. It is obvious that these vessels, normally undergoing regressive changes on account of the lack of sufficient hydrodynamic force to maintain their circulation, will not remain patent unless the circulation through them is maintained. In this connection the retention of the function of hæmatopoiesis is significant. The continual formation of blood cells, if present, may supply the *vis a tergo* necessary to keep these vessels open. The same factors at work in the normal obliteration of these vessels may be active and offer some hindrance to the egress of blood elements formed *in situ*, and in this way tend to maintain a higher pressure with resulting distention. The well-known fact of the great increase in resistance produced by the insertion of angles or elbows in an hydraulic system is applicable, and the difficulties these angles may offer in the way of satisfactory injection experiments is obvious.

In the absence of hæmatopoiesis, resistance to the outflow (*e. g.*, by angles) or forces similar to those in operation at the nodal points of the liver may be present, and it seems possible that a study of the architecture of angiomata by means of corrosion specimens with injection of the general circulation and of the angioma, as well as a similar study by various reconstruction methods, may demonstrate the presence of sufficient mechanical factors to explain the occurrence of these structures.

It seems probable that angiomata may be explicable on this basis, and that the cases showing real growth of blood vessels may be the result of a secondary change in the walls of a simple hæmangiectatic structure.

Summary

1. Diffuse hæmangiectases of the liver are rare and the association with a similar process in the kidneys and adrenal glands as reported in this paper has not been previously described.

2. Hæmatopoiesis is not infrequently observed and may be present in all angiomata. Further confirmation of this point is desirable.

3. The early vascular system develops in the form of a capillary plexus. The subsequent growth of certain capillaries and the regression of others are controlled by the laws of mechanics.

4. The persistence of capillaries which normally undergo regressive changes offers an explanation for the occurrence of hæmangiomata or hæmangiectases.

To prove this theory it is necessary to determine what forces are present to maintain the patency of these vessels. Hæmatopoiesis may supply the necessary force. Furthermore, other mechanical factors may be important. In order to clarify this point, it is desirable to determine whether blood formation is present in all hæmangiomata and to study these structures by injection and by reconstruction methods.

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FIG. 1.—Diffuse hæmangiectasis of the liver. A section showing the periportal distribution of the dilated sinuses. (× 55.)

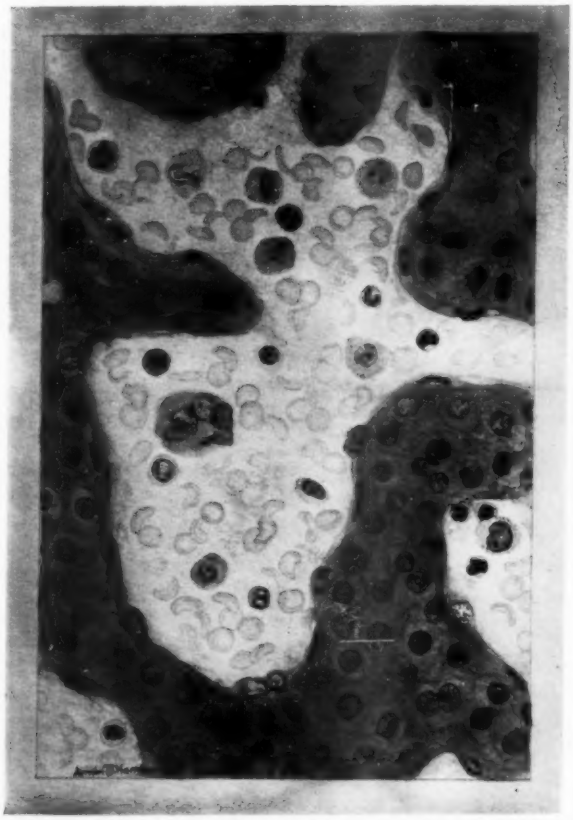

FIG. 2.—Diffuse hæmangiectasis of the liver. A high-power drawing of a dilated sinus, showing nucleated red blood cells and immature cells of the myeloid series as evidence of hæmatopoiesis. (× 800.)

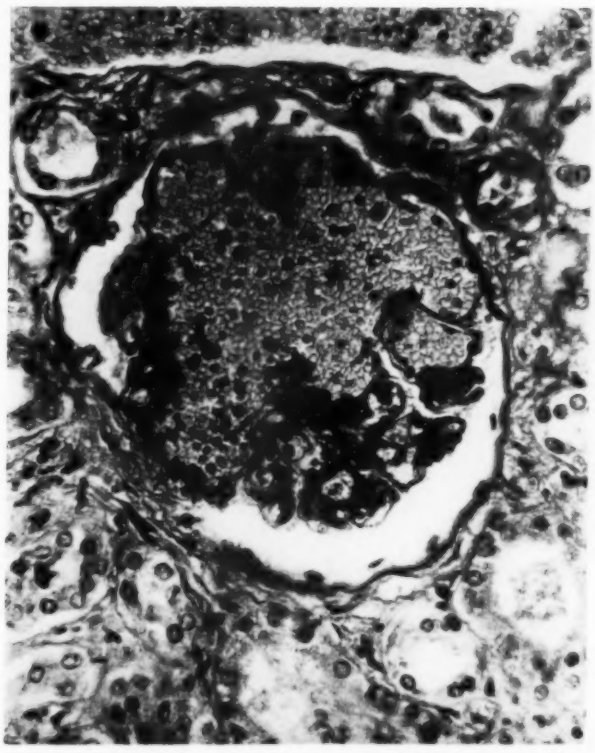

FIG. 3.—Diffuse hæmangiectasis of the kidneys. A section showing extreme dilatation of the vessels of a glomerular tuft. (× 375.)

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CHEMICAL STRUCTURE AND PHYSIOLOGICAL ACTION*

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In my previous lecture I asked your attention to the available evidence as to the intimate mechanism of the anaphylactic shock. There are many broader considerations of the meaning of the anaphylactic condition which are worthy of attention, but I can touch upon a few only. From the teleogical point of view, the phenomena present at first sight some difficulty. Of what value can it be to the individual, or to the species, that one injection of a harmless substance should be followed by such a change in the constituents of the cells or the body fluids that the same substance, if again injected, acts as an acute, often as a lethal, poison?

The meaning begins to be clearer when we recognize that anaphylaxis is not, as its name was intended to imply, the opposite or antithesis of immunity, but a phase in the development of immunity, in which the immune substance or antibody has a peculiar distribution. We must recognize, further, that the danger to the individual of the anaphylactic state is largely the effect of the purely artificial conditions which we impose by the use of the hypodermic needle, by means of which we bring a foreign protein into the circulation with a rapidity which no natural method of absorption could achieve. Fundamentally the immune reaction, of which anaphylaxis is a specialized phase, represents the need of the species to prevent the permanent incorporation into the cells of protein having a different type from its own. That such incorporation, at least into the surface of the cell, may temporarily occur, is demonstrated by certain of the phenomena of anaphylaxis itself. We have seen how the precipitin from the serum of an immunized rabbit can be taken up by the cells of a guinea-pig, which is thereby rendered passively anaphylactic to the substance against which the rabbit had been immunized. Weil demonstrated that the passive anaphylaxis thus conferred lasted only for eight or ten days, at the end of which time its disappearance coincided with the appearance of a new, active anaphylaxis to rabbit protein. The guinea-pig, in thus becoming anaphylactic, has rid its cells of the rabbit protein, which they had for the time incorporated, and prepared them to deal more rapidly with any new intrusion of the same protein. If the next invasion is sufficiently sudden and massive, the shock of the resisting mechanism, dangerously located to meet such attack in the vital cells of the organism, may lead to the death of the individual. With successful resistance to a series of milder and more gradually developed attacks, such as the organism is likely to meet under natural conditions, as contrasted with those created by the injection needle, the defensive mechanism is strengthened and extended beyond the cells, and the immune body circulates in sufficient excess in the blood to meet the invading foreign protein and secure its harmless removal from the sphere of action. A true immunity has been established.

In the anaphylactic shock we may contemplate the sacrifice of the individual to the purity of the type. Note that it is the *proteins* in particular from other species, against which this defensive system of anaphylaxis and immunity is prepared. It is the proteins which give to the species its distinctive biochemical character. The same fats and carbohydrates are found in a multitude of different types; the composition of an animal's body fat may change to some extent with the fats supplied in the food. According to recent evidence, it would appear that the lipoids and even the nucleic acids have the same composition through a wide range of species. The carbohydrates and fats are fuel supplying energy; the lipoids and nucleic acids seem to have the function of conferring on the cell protoplasm the physical characters on which its vital activities depend and of furnishing the supporting framework of the structures with which the physiological functions of the cell are inseparably connected. But, as Loeb and Levene have recently pointed out, it is on a difference of the proteins that the difference between species depends. One cannot imagine any replacement of the proteins of one species with those from another without loss of the specific character.

Against such intrusion the unicellular organism can apparently guard itself by the aid of its proteolytic ferments. In the higher animals this power of digesting foreign proteins is wanting, or held in abeyance, in the ordinary cells of the body, and the function is concentrated in the cells of the digestive glands. Ordinarily the digestive juices afford efficient protection to the body against the introduction of proteins foreign to its constitution, splitting the proteins of the food into their constituent amino-acids, from which, in

* Lecture III of the Herter Series, delivered before The Johns Hopkins University on Nov. 15, 1919.

† It should be noted that in the lectures published in the August and September numbers, Dr. Dale was down as representing the Lister Institute of Preventive Medicine, which was incorrect.

due course, proteins with the architecture characteristic of the species can be built up. Occasionally the protection fails, as in the cases of alimentary anaphylaxis. As a rule, however, it is only when the foreign protein is introduced by some other route than that of the alimentary canal, that it passes uncloven into the blood stream and calls into action the secondary provision for its ultimate elimination. Nor is it only the proteins from *foreign* species which meet with this opposition to their permanent incorporation into the body cells in general. In some cases proteins from certain organs of the animal's own body, being presumably of a specialized type, are treated as foreign to the rest of the body, if they abnormally pass into the general circulation, and evoke a response of the mechanism of anaphylaxis and immunity.

It would lead me far beyond the limits of my time, and of my competence, to attempt to deal with the relation between anaphylaxis, seen in its simplest form in experiments with pure proteins, and the physiological changes accompanying the body's resistance to foreign protein introduced more gradually into the tissues of the circulation by the growth of microörganisms. That there is a very suggestive connection between some of the phenomena constituting the clinical disease produced by infection, and those of the anaphylactic reaction as seen in experiment, is, I think, abundantly clear, and it has received emphasis in the writings especially of Vaughan, Friedberger, and more recently Novy.

I must be content, moreover, with a mere reference to the much clearer connection between experimental anaphylaxis and certain natural or acquired idiosyncracies to foreign protein, whether taken in the food, as in the fairly numerous well marked cases of intolerance for egg-protein which are on record, or absorbed through the respiratory mucous membrane, as in the cases of intolerance for the exhalations of certain animals, or the pollen of certain plants.

I want to ask your attention rather to what is at present the more academic problem of the nature of the difference which makes the protein of one species *foreign* to, that is to say, unsuitable for incorporation into the tissues of another. What is the nature of the difference which the anaphylactic or precipitin reaction detects in proteins which, to ordinary methods of appreciation, are so strikingly similar?

I may remind you that the protein molecule is built up from a number of different amino-acids, linked together into peptide chains, which chains, being again linked into an intricate skein, build up a molecule of immense complexity. We may distinguish three kinds of difference, which the reaction of immunity or anaphylaxis can detect.

1. There are cases in which a difference of antigenic properties can be related to a difference of chemical constitution so crude that a mere hydrolysis of the two proteins into their constituent amino-acids suffices for its detection. The different amino-acids are found to be present in widely different proportions in the two proteins; certain amino-acids may be present in the one which are not represented at all in the constitution of the other. The two proteins are obviously different chemical substances, and the difference is revealed by a relatively superficial analysis. While it may be said, on the one hand, that no difficulty of conception is presented by the fact of their different antigenic properties, it does not, on the other hand, give us any clue to the nature of the relation between antigen and antibody which thus discriminates between them. As an instance of antigenic disparity between proteins having such obvious difference of constitution, we may take the case of the albumin and globulin from the same serum.

When analyzed into their amino-acids these at once show a pronounced difference of structure; the albumin, for example, contains much more lysine than the globulin, while glycine, which is well represented in the globulin molecule, appears to be absent altogether from that of the albumin. Hartley and I found that the pure albumin and euglobulin, which he had prepared from horse-serum, acted as completely distinct antigens. Not only did the guinea-pig sensitized to euglobulin

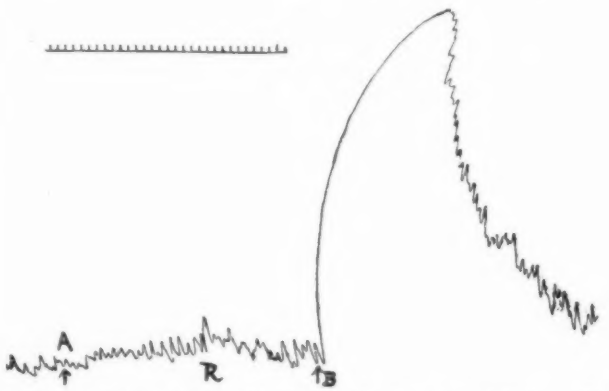


FIG. 1.—Guinea-pig sensitized with 1 mgm. of euglobulin. 17th day. *A*, 5 mgm. of albumin. *B*, 1 mgm. of euglobulin. (Dale and Hartley, Biochemical Journal, Vol. X, p. 421, Fig. 5.)

fail to react to albumin, and *vice versa;* in the guinea-pig rendered sensitive with whole horse-serum anaphylaxis to euglobulin appeared and attained its maximum, much earlier than that to albumin. (See Fig. 1. The tracing records contraction of the isolated uterine plain muscle.)

2. The two globulins of a serum—the so-called euglobulin and pseudoglobulin—present a difference of another kind. They are built up of the same amino-acids in apparently identical proportions, and even the finer method of structural investigation, presently to be discussed, fails to detect any difference between them. They show, however, a pronounced difference in their physical properties and solubilities, the euglobulin being quite insoluble in pure water and needing the presence of acid, alkali and a neutral salt to disperse its aggregates and bring it into colloidal solution, while the pseudoglobulin is taken up by pure water like an albumin. This difference between the globulins appears to be due to the fact that, in the euglobulin, a phosphorus-containing lipoid is associated with the protein. The physical difference thus conferred is again associated with an antigenic disparity. Hartley and I found that a guinea-pig rendered sensitive

to the euglobulin of horse-serum was not sensitive to the pseudoglobulin (Fig. 2). The fact that guinea-pigs sensitized to the pseudoglobulin showed a minor degree of sensitiveness to the euglobulin also, is probably to be attributed to the extreme difficulty of purifying the pseudoglobulin completely from residual traces of euglobulin.

3. The most interesting cases, however, and till quite recently the most mysterious, are those in which neither an ordinary analysis into amino-acids, nor an examination of the physical properties, reveals any difference between two proteins, which are shown by the immunity reactions to be

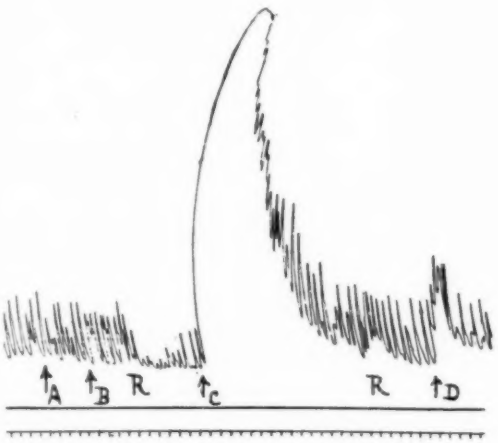


FIG. 2.—Guinea-pig sensitized with 1 mgm. of euglobulin. 13th day. *A*, 1 mgm. of pseudoglobulin. *B*, 10 mgm. of pseudoglobulin. *C*, 10 mgm. of euglobulin. *D*, 10 mgm. of euglobulin. (Dale and Hartley, Biochemical Journal, Vol. X, p. 422, Fig. 6.)

different substances. Abundant examples can be found in corresponding proteins from species not too widely separated—caseins from cow's and sheep's milk, pure albumins from hen's and duck's eggs, or corresponding proteins from plants of the same family. No chemist by identifying the nature and estimating the proportion of each amino-acid present can distinguish one member of such a pair from the other, but the anaphylactic reaction detects a difference where, until recently, chemistry was at a loss. There were those who supposed that the disparity depended on some physical difference in the structure of the colloidal solutions; others were ready to attribute it, not to the pure proteins, but to traces of associated lipoids; in both cases without any evidence.

There was always another possibility open, however, namely, that the antigenic individuality depended not merely on the nature and preparation of the amino-acids in the molecule, but on the order of their arrangement into the intricate molecular pattern. If one takes a heap of counters, comprising, say, a dozen different colors, with many of each color, it is possible to make an enormous number of different color patterns, without varying the number of colors or the proportions in which they are represented. Similarly, it is possible to imagine an almost endless variety in the intimate pattern of the protein molecules which can be constructed from the same amino-acids in the same relative proportions. Only in the last few years has it become possible, through the work of Dakin and Dudley, to obtain some insight into the existence of this type of structural difference among proteins. With the single exception of glycine, all the amino-acids are constitutionally asymmetrical substances and exist in the protein molecule in the optically active form. The whole protein molecule, accordingly, is optically active, rotating the plane of polarised light. Kossel had shown that, under certain treatment, this optical activity of a native protein was gradually reduced to a steady minimum, and a first indication of the close relation between antigenic properties and the stereochemical structure was obtained, when Ten Broeck took such racemised egg-white, which Dakin and Dudley had prepared by Kossel's method, and found that it neither caused any sensitiveness in a guinea-pig, nor provoked any anaphylactic reaction in a guinea-pig rendered sensitive to the normal unracemised protein. Dakin and Dudley had already found that racemised protein was completely resistant to proteolytic ferments. It behaved in the body, indeed, not like a protein at all, but like a completely inert and indifferent material.

When such a racemised protein is hydrolysed into its constituent amino-acids, it is found that many of the amino-acids have been racemised completely, but that some retain partly or wholly their native optical activity. From a study of the behavior of certain analogous complexes, Dakin found reason for believing that the groups thus escaping racemisation are those which form the terminal links of the peptide chains out of which the molecule is built.

Here, then, was a method for obtaining a first hint as to the existence of structural difference between otherwise indistinguishable proteins. Dudley and Woodman first applied it to the caseins of cow's and sheep's milk, and found that, while these two proteins consist of the same amino-acids in identical proportions, the groups remaining optically active, when the whole protein is racemised as far as possible, are not identical in the two cases. The difference was small, but definite. It seemed desirable to repeat this comparison on two corresponding proteins having pronounced antigenic properties, and Dr. Dakin and I arranged a joint experiment. Similar proteins were to be taken from different species; Dakin undertook a comparison of their structure, in so far as his method would reveal it, while I undertook to investigate their antigenic specificity by the anaphylactic reaction. We chose the crystalline egg-albumins of the hen and the duck, partly because they were easily obtained pure, but chiefly because some preliminary experiments by Hartley and myself had appeared to indicate that they were indistinguishable, even by the anaphylactic reaction. Dakin, however, found a clear indication of difference in the order of arrangement of the amino-acids in the two albumins, and, on making more thorough and careful experiments on their antigenic properties, with samples of the pure products used by Dakin for his analysis, I found that the anaphylactic reaction distinguished clearly between them, though not with

absolute specificity. Table I summarizes the results of Dakin's examination of the changes in optical rotation of the individual amino-acids, when the two albumins were racemised.

TABLE I

Amino-acid	"Racemised" hen albumin	"Racemised" duck albumin	Comments
Alanine (1).	Not racemised.	Not racemised.	No difference.
Valine (2).	Partly racemised.	Partly racemised.	" "
Leucine (3).	Mostly racemised.	Mostly active.	*A definite difference.*
Proline (4).	" "	Mostly racemised.	No difference.
Phenylalanine.	Completely inactive.	Completely inactive.	" "
Tyrosine (5).	Inactive.	Inactive.	" "
Aspartic acid (6).	Mostly inactive, some active.	Completely inactive.	*Definite difference.*
Glutamic acid.	Completely inactive.	" "	No difference.
Histidine (7).	" "	Mostly active.	*Definite difference.*
Arginine.	Active.	Active.	No difference.
Lysine (8).	Inactive.	Inactive.	" "

(From Biochemical Journal, Vol. XIII, p. 250)

Fig. 3 shows the response of the isolated uterine plain muscle of a guinea-pig, sensitized by a preliminary injection of hen albumin. It will be seen that it is indifferent to 2 milligrams of duck albumin, but responds by maximal tonus to 0.1 milligram of hen albumin, to which it is thereafter completely

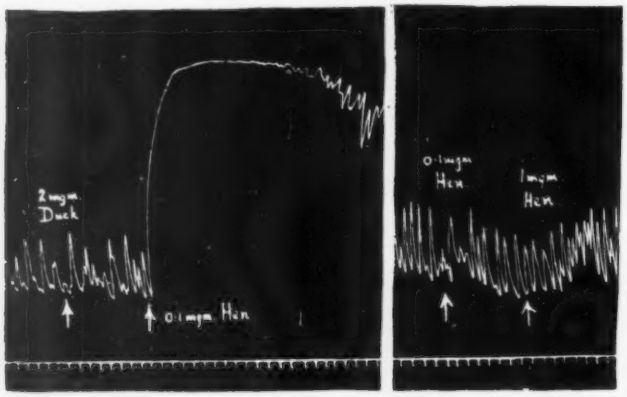


FIG. 3.—Sensitized with 1 mgm. of hen albumin. 21st day. (Dakin and Dale, Biochemical Journal, Vol. XIII, p. 253, Fig. 1.)

desensitized. Figs. 4 and 5 show similar exeriments on the two horns of the uterus of a guinea-pig similarly sensitized to duck albumin. It will be seen (Fig. 5) that there is, in this case, a small response to 2 milligrams of hen albumin, but a subsequent maximal response to 0.1 milligram of duck albumin. The sensitization is not absolutely specific but very highly preferential. The reactions make a perfectly clear but not absolute distinction between the two proteins.

It is obvious that Dakin's method does not reveal the whole of the difference in structure which may exist between the two molecules. We are given, as it were, an imperfect glimpse of the edge of the pattern; but a difference already visible in such a partial view is the more significant. The window thus opened is in itself a small one, but it looks on a wide vista of possibilities.

I think we are entitled to hope that it will eventually be possible to state antigenic specificity in terms of structural chemistry, to obtain a clear view of the intimate molecular pattern which gives to the protein its individuality, and of

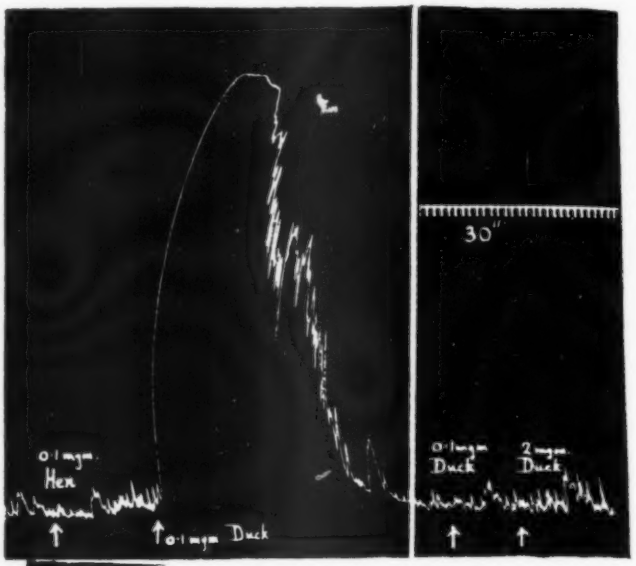


FIG. 4.—First horn. Sensitized with 1 mgm. of duck albumin. 28th day. (Dakin and Dale, Biochemical Journal, Vol. XIII, p. 254, Fig. 3.)

which the relation to a particular antibody has hitherto been the only indication. Perhaps it is not beyond reasonable hope that the antibody also may be obtained in sufficient purity

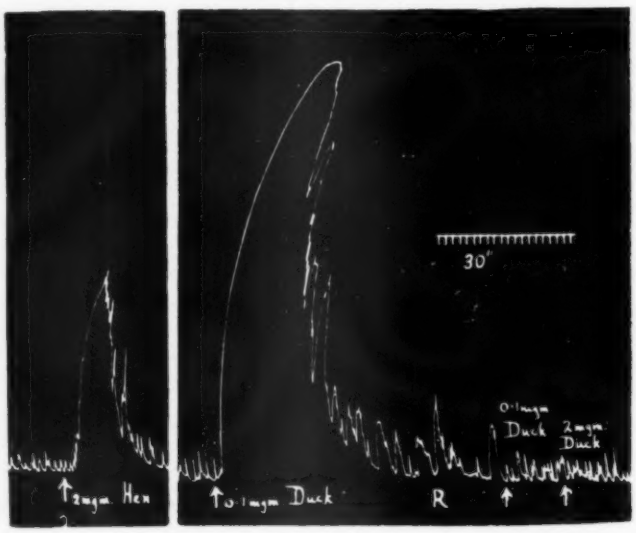


FIG. 5.—Second horn. (Dakin and Dale, Biochemical Journal, Vol. XIII, p. 255, Fig. 4.)

for such investigation of its structure as will enable us to replace, by a rational conception, the pictorial schema of its relation to the antigen, which Ehrlich displayed in the side-chain theory.

As to the nature of that relation, I think it must be admitted that exact knowledge is, as yet, almost entirely wanting. We are dealing with substances of very great molecular dimensions, and these molecules are so aggregated that they form typical colloidal solutions. It seems inevitable that surface phenomena will play a part in their interaction and formation of compounds, and may well impose upon the union quantitative relations which, viewed by themselves, would give to the whole process the aspect of an adsorption. But any such purely physical hypothesis will be inadequate. There has been an attempt to arrive at a simple electro-chemical conception of the union between antigen and antibody, representing it as the discharge and mutual precipitation of oppositely charged amphoteric colloids. Without denying the possibility that such a process may participate in some kinds of immune reaction, it is evident, I think, that these simple physical conceptions fail altogether to account for that specificity of discrimination, that exquisite adjustment of the antibody to a particular structure of the antigen molecule, which is the characteristic and essential feature of the relation. On the other hand, the existing chemistry does not enable us to account for this on purely chemical lines. It is hardly credible that it should involve a chemical combination in the sense of a synthesis by replacement. It must rather belong to the type of those molecular combinations, the dividing line between which and adsorption compounds is at present but ill-defined. We can go little further as yet than the citation of imperfect analogies. We have, for example, the relation of an enzyme to its substrate, again intimately dependent on the structure, and on the optical asymmetry of the substrate molecule, but presenting features which make it practically certain that surface phenomena play a part in bringing the two into relation. The analogy, however, is by no means a close one; it presents no parallel to the nicety of specific adjustment of the antibody to the antigen.

There is another comparison which, in this direction, shows us a more suggestive similarity, and which almost forces itself on the attention of any one who has been concerned with both types of investigation; namely, that between this union of antigen and antibody and the relation of certain classes of drug substances to the tissues or organs on which their action is specifically localized. You will remember that a comparison and contrast of the action of antigens with that of drug substances played a prominent rôle in the genesis of that wonderful fabric of theory with which Ehrlich so largely influenced the form and direction of research in pathology and pharmacology for almost a generation.

Nothing is further from my intention than to attempt here a survey of the enormous body of work and speculation which has been devoted to this ultimate pharmacological problem, of the relation between the chemical structure of a substance and its pharmacological action. It appears to me that we must recognize the improbability that the whole of the widely different types of activity of chemical substances will ever be brought under one principle of interpretation; indeed, I believe that progress towards rational conceptions has been definitely retarded by attempts to force all kinds of pharmacological action under schemes of explanation which are found to have some application to one type in particular. For example, the relation between the activity of a large group of narcotic and anæsthetic substances and their solubility in fats and lipoids was realized by Ehrlich and presented with much suggestive detail of evidence by Meyer and by Overton. The significance once generally accorded to the high partition coefficient of these substances for lipoids, as compared with water, has been weakened by later evidence showing a similar relation between their narcotic action and other physical properties—their effect in lowering the surface tension of water, in aggregating protoplasm, in affecting the rate of gelation of certain colloidal systems, and so on. Possibly some of these may prove to be different aspects of the same fundamental property. There is evidence pointing to adsorption, as well as preferential solution in lipoids, as playing an important part in the concentration of these substances out of the blood into the cells whose activity they depress. But, whatever may be the ultimate conclusion as to the exact mechanism of the action of substances of the class of alcohol, chloroform, ether, I think it is reasonable to expect that it will be found to have a relatively simple physico-chemical basis.

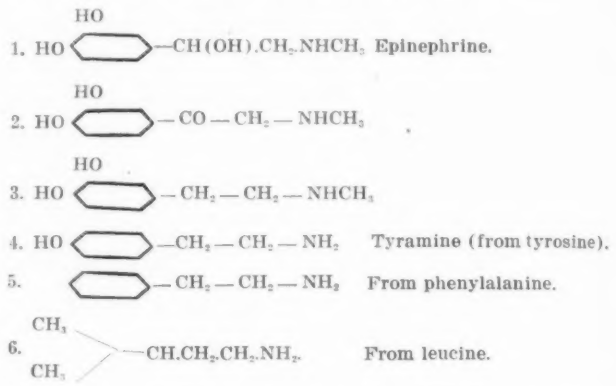
There has been some danger, however, lest such simple physical conceptions should be given a width of application which the evidence will not justify; lest they should be invoked, with a facile vagueness, in explanation of certain types of activity which are characterized by an extraordinary precision of localization and a close dependence on certain types of chemical structure. It is here that we encounter, as it seems to me, many suggestive points of analogy with the relation between antigen and antibody, and it is of a few examples, which happen to have come within the scope of investigations in which I have taken part, that I wish now to speak.

The types of action which I propose to mention are all exhibited by alkaloids and organic bases. No neutral or acidic substance, to my knowledge, has been shown to have a strictly localized action of the type I have in mind. The localization is so limited and exact that the action affects only certain functions of cells receiving their innervation from a particular morphological division of the nervous system.

An early example of such a precisely localized action was the paralyzing effect of curare, located at the end plates, where motor nerve-fibres end on skeletal muscle-fibres. Fraser and Crum Brown showed the close association between this type of action and the structural type of the quaternary ammonium-base. The parallel, however, is not complete; there are other substances than ammonium bases which have a curare-like action, such as cobra-venom; and there are certainly ammonium bases having a different type of action, resembling that of nicotine, which has a stimulant action localized on ganglion cells and, in varying degrees, on skeletal muscle fibres, and which, as Langley has shown, in general stimulates where curare paralyzes. Then there was the peripheral paralysis by atropine and hyoscine, and by many artificial esters of tropine, of the effects of nerves belonging to the cranial division

of the involuntary or autonomic system—the vagus, the chorda tympani, the short ciliary nerves; and the stimulant effects of pilocarpine, arecoline and muscarine, localized exactly where atropine and hyoscine paralyze.

Interest in such localized actions received a great impetus when it was discovered that an alkaloid occurring naturally in the body, and secreted continuously into the blood, the hormone-alkaloid epinephrine, had an action thus localized with extraordinary precision, as indicated by Lewandowsky and Langley, and demonstrated with a wealth of detailed evidence by Elliott and many later workers. The action of epinephrine, strictly localized at the periphery, reproduces with remarkable fidelity the action of nerves of the true sympathetic system and of no others, augmenting where these augment, inhibiting where they inhibit, and leaving altogether unaffected involuntary muscle and gland cells having no true sympathetic nerve-supply. It does not act on the nerves, since its action survives and is even intensified by complete degeneration of these to their ultimate visible terminations; nor does it act directly on the contractile or secretory elements in the effector organ, since its action is extinguished by the action of certain poisons, which also block the passage of impulses from sympathetic nerves, but leave the muscle or gland cell normally responsive to other kinds of stimuli, whether nervous or chemical. Such poisons render the muscle or gland cell irresponsive to sympathetic nerve impulses and to epinephrine, but leave them in other respects normal. Atropine similarly renders cells specifically indifferent to pilocarpine or muscarine. There is one such poison which differentiates function even more finely, eliminating the motor effects of sympathetic nerves and of epinephrine, but leaving the cells responsive to their inhibitor actions and to both motor and inhibitor stimuli of other kinds. This is the alkaloid of ergotoxine, the first of the pharmacologically interesting substances yielded by ergot, and the only one peculiar to it. There was at one time a tendency to cut the knot of the problem presented by the resemblance between the action of epinephrine and the effects of sympathetic nerves, by boldly assuming that the nerves of this system produce their effects by liberating epinephrine at their peripheral terminations. And certainly it is remarkable that a substance produced from the cells of the suprarenal medulla, which embryologically have the same origin as sympathetic ganglion-cells, should have its action localized on structures in which the axons of those ganglion-cells terminate at the periphery. But we are reminded of the theory which would explain the anaphylactic shock by the liberation of some substance of the histamine type. As in that case the pathologist, in this the physiologist seeks to get rid of his problem by reducing it to terms of pharmacology. Again, however, it seems to me that this merely shifts the incidence of the difficulty, without in the least degree removing it. It does not tell us why epinephrine, and, as we shall see, substances resembling it more or less closely in structure, when injected into the general circulation, have their action localized at the peripheral distribution of sympathetic-nerve fibres. We must suppose that there is some structure which acts as transmitter between nerve fibre and contractile or secretory element, which determines the effect of the nerve impulse in the direction of augmentation or inhibition; and that in this myoneural or cytoneural junction there is some substance having a specific affinity for epinephrine and for substances of similar structure. Our problem is to discover what can be known of the nature of such a substance, and of the nature of its affinity for these bases.


1. HO —CH(OH).CH₂.NHCH₃ Epinephrine.
2. HO —CO—CH₂—NHCH₃
3. HO —CH₂—CH₂—NHCH₃
4. HO —CH₂—CH₂—NH₂ Tyramine (from tyrosine).
5. —CH₂—CH₂—NH₂ From phenylalanine.
6. CH₃ CH₃ —CH.CH₂.CH₂.NH₂. From leucine.

I have written here the formulæ of six substances exhibiting in greater or less degree this epinephrine-like action, which Bayer and I termed "sympathomimetic" action. No. 1 is epinephrine itself; 2 is a synthetic compound from which epinephrine can be made artificially by reduction; 3 is also an artificial product of synthesis; the rest are bases derived, like histamine, from amino-acids, and therefore formed by the action of certain bacteria on products, and found in ergot and other fungi.

What is to be learned from a series like this, as to the nature of the affinity which so specifically localizes their action?

I do not think the conception of a specific solvent, extracting these bases preferentially from the blood, can really help us here. The lower members of the series are perfectly miscible with water, but can be extracted from it by organic solvents, in which they are even more soluble. Epinephrine and its immediate homologues are curiously insoluble in practically all neutral solvents. There are intermediate terms between these extremes of the series, but there is nothing to indicate a gradation of activity corresponding to any special kind of solubility. Nor is there any other physical property which appears to show any parallelism with activity.

There is, again, no kind of chemical reactivity which seems to be graded in the series in accordance with intensity of action. Reducing action has been mentioned as a characteristic chemical property of epinephrine, and attention has been drawn to the increase of both physiological activity and reducing action in passing from the ketones to the alcohols, as from 2 to 1 in the series given above. But there is another change effected in reducing the ketone to the alcohol, which has probably greater significance, namely, the introduction of an asymmetrical carbon atom and the production of an optically active compound. The reducing action of the ketones can be

similarly augmented by introducing a third phenolic hydroxyl, making a pyrogallol derivative, but this is slightly less, instead of much more, active than the parent catechol derivative; also it has still an optically symmetrical structure.

The only strictly chemical property common to all these substances is that they are bases. I think it is quite likely that this property is related to the fact that they are physiologically active on the cells in which they become locally concentrated. But for the reason of this localization and concentration, on which the specific nature and intensity of the action must primarily depend, we must look elsewhere.

I think there is no escape from the conclusion that the action is not associated with any physical characteristic or type of chemical reactivity which present knowledge enables us to appreciate or measure *in vitro*. It is associated rather with a type of chemical structure which gives to the substance a specific relationship to some substance in the myoneural junction; a relationship which becomes increasingly specific as the structure of epinephrine is approached. Again, as in the relation of antigen to antibody, we seem to be concerned with a relation of which the basis is chemical, as shown by its intimate dependence on structure, which cannot, however, be regarded as the synthetic formation of a new firm compound by replacement; which must be some looser type of additive molecular combination. Since we are dealing with concentration in, or on, the surface of living cells, we are dealing with a complex colloidal system, and it is highly probably that surface phenomena play a part. But, if we regard the relation as an adsorption, it must be an adsorption of a highly specific kind, closely dependent on the chemical structure as well as the physical nature of the surfaces concerned. Of such an adsorption, specifically conditioned by chemical structure, the physical chemist at present can tell us almost nothing. We are again in the borderland between chemical and physical union, the exploration of which holds out such promise for the illumination of biological conceptions.

Passing down the series as I have written it above, we see how a relationship, most intense and specific in the case of epinephrine and its immediate homologues, gradually becomes weaker and less specifically localized as one group after another is removed from the epinephrine molecule. In passing from epinephrine 1 to 2 or 3 the alcoholic hydroxyl of the side chain goes, optical asymmetry disappears and the action is weakened. In the lower members of the series the methyl group has disappeared from the amino-radical, and in these simpler compounds its absence seems to make no material difference to the activity. It is otherwise in the case of 1, 2, and 3. The primary amine corresponding to epinephrine, but having $-NH_2$ in place of $-NHCH_3$, has a more powerful pressor action than epinephrine itself, and the same is true of 2 and its primary homologue; on the other hand the primary amine corresponding to 3 is much less active than the latter. It is a point of curious interest that the $-NHCH_3$ compound is in all cases much more potent in reproducing *inhibitor* sympathetic effects.

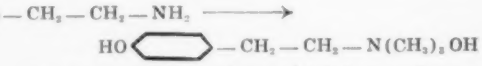
These relations, however, are rather too complex for our present purpose, which is to note how with successive departures from the epinephrine structure, removal of first one phenolic hydroxyl (in 4), then of the second (in 5), and lastly with disappearance of the benzene ring (in 6), we find a progressive weakening of the sympathomimetic action and progressive lessening of its specificity. Larger and larger doses are needed to evoke the characteristic effects, and they are more and more complicated by effects of other types. No. 6 has hardly any longer a perceptible resemblance to the epinephrine structure; it is, as it were, a mere featureless stump from which the characteristic outlines have been cut away. It still has a very definite sympathomimetic action, though weak and poorly specific. If we adopt Emil Fischer's comparison of the relation between enzyme and substrate as the fitting of a key into a lock, we may say that, in this series, as each ward is filed off the key, the fit becomes ever looser; it turns the lock with increasing difficulty and begins to fit others, though not well.

Keeping to the figure for a moment—and remembering that it is nothing more than a diagram—we may inquire what happens if, instead of removing wards, we add an excrescence to those present. Apparently we may destroy the fit of the key entirely. As the phenolic hydroxyls are removed we have seen that the activity becomes weaker and less specific; if they are methylated, replacing HO— by CH_3O—, the activity disappears completely.

The comparison with the relation of an enzyme to its substrate is rendered the more apposite by the discovery that the optically opposite forms of an asymmetrical compound, like epinephrine itself, differ very widely in their activities. Cushny found the natural *l*-epinephrine to be many times as active as the artificial *d*-epinephrine, just as he had found a similar difference of activity between the two hyoscyamines, and more recently between the two hyoscines. Similarly Dakin showed, many years ago, that, of two optically isomeric mandelic esters, both were hydrolized by a lipase, but one with much greater rapidity than the other. He interpreted this result as indicating that the enzyme was itself asymmetrical and formed additive compounds with the two isomers, which would not then be optical antipodes. Cushny has similarly suggested the probability that the receptive substance, which localizes the action of an optically active drug-substance, is itself asymmetrical in structure. Whether the greater activity of lævo-epinephrine should be attributed to its more rapid entry into molecular combination with this substance, or to the more rapid occurrence of some consequent change, as Dakin suggested in the cases of the hydrolysis of the mandelic esters, is a point on which we have no basis of evidence even for surmise.

Let me mention briefly another series of compounds, having an activity with an entirely different localization from that which we have been considering. I have mentioned already the classical observation of Crum Brown and Fraser as to the curare-like action of quarternary ammonium bases. If we make an ammonium base from any member of the epinephrine series, by completely methylating the amino-group,

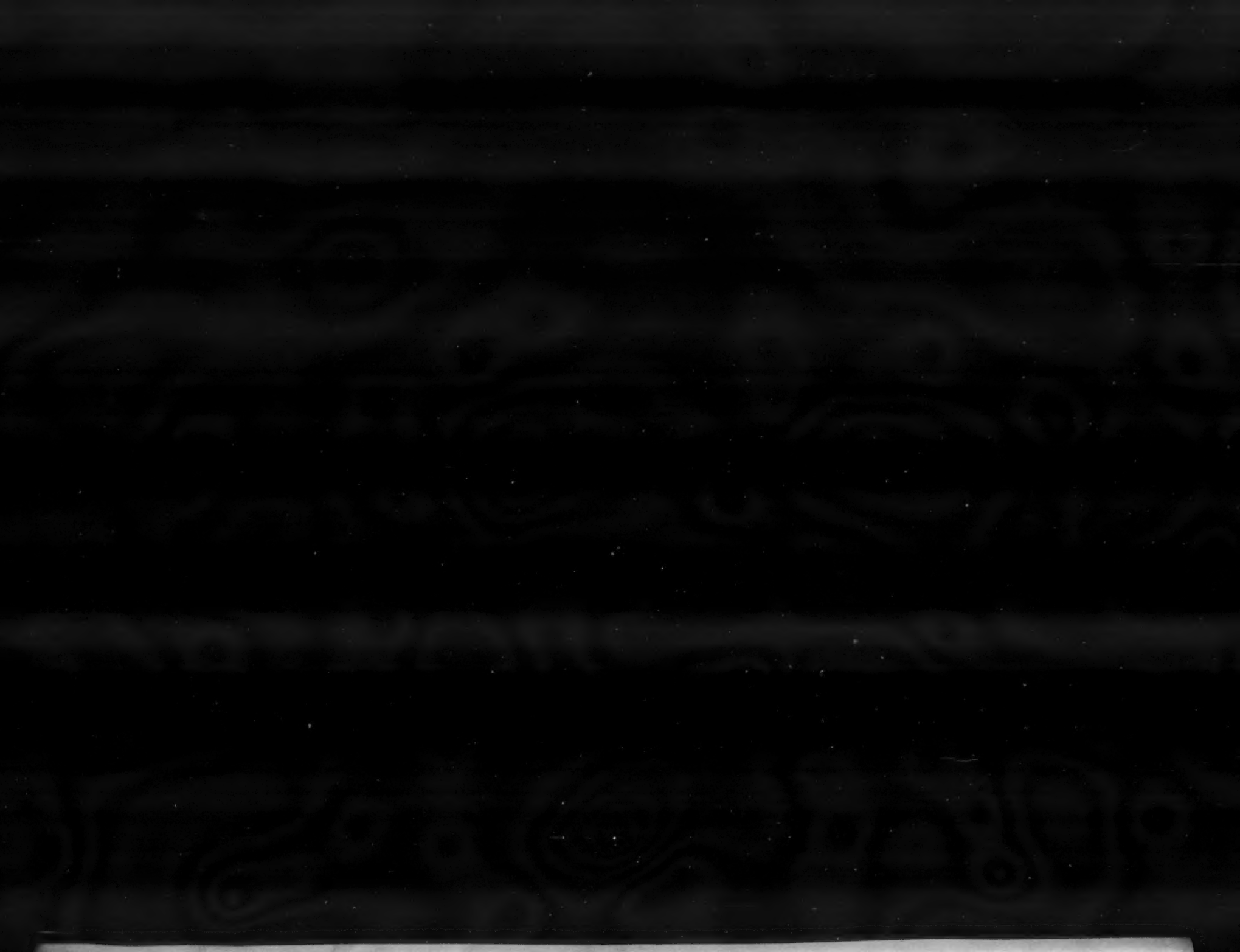
we obtain not a curare action, but a powerful action combining effects of nicotine with those of muscarine, though these latter are but weakly represented. If we take, for example, No. 4 of the above series (tyramine) and methylate its amino-group completely,



we obtain a quaternary ammonium base which has no longer a sympathomimetic action, but in its place a powerful stimulant action on autonomic ganglion cells and skeletal muscle fibres, closely resembling that of nicotine, and in addition a weaker peripheral action of the muscarine type, related to innervation, not by sympathetic nerves but by nerves of the other divisions of the autonomic system. We see a new significance in this change if we consider the molecule, as it were, from the other end, and realize that this quaternary compound may be regarded as a derivative of choline. It appears as No. 4 of the following series of derivatives of choline. Choline itself has, in a very weak form, both the nicotine and muscarine types of action, and both are intensified, in different degrees, by various forms of substitution of the alcoholic hydroxyl.

1. $HO(CH_3)_2N-CH_2-CH_2-OH$ Choline.
2. $HO(CH_3)_2N-CH_2-CH_2-OC-CH_3$ Acetyl-choline.
3. $HO(CH_3)_2N-CH_2-CH_2-ONO$ Nitroso-choline ("synthetic muscarine").
4. $HO(CH_3)_2N-CH_2-CH_2-C_6H_4-OH$ Trimethyl tyramine.
5. $HO(CH_3)_2N-CH_3$ Tetramethyl ammonium hydrate.

In No. 2, acetyl-choline, the muscarine-like action is of unparalleled intensity, as shown by Reid Hunt and myself. The dose which will cause a perceptible fall of arterial pressure in a cat is of the order of 10^{-8} milligram. The substance, however, is so rapidly hydrolysed in the body that the action of even larger doses is extremely evanescent. No. 3 has so pronounced a muscarine-like action that it was long believed to be an artificial muscarine, till Ervins and I showed that the action of nitric acid on choline is not to oxidize it but to form this nitroso-compound. Both 2 and 3 possess also a nicotine-like action, much more powerful than that of choline, but normally masked by their predominant muscarine effect. In 4 the nicotine-effect becomes predominant.

Again we are at a loss to relate the independent variation of these two types of action to any recognizable chemical or physical properties; and again we find them recognizable in the simplest remnant, as it were, of the molecule. No. 5, tetramethyl ammonium hydroxide, with the side chain reduced to an additional methyl group, still has both types of action, the nicotine action with great intensity. And in further illustration of the difficulty of the problem, let me draw your attention to the remarkable contrast between the action of this substance and its tetra-ethyl homologue, $HO(C_2H_5)_4N$. In all ordinary chemical and physical properties the tetramethyl and tetra-ethyl ammonium salts are practically identical. Yet the tetramethyl ammonium salts have these two types of activity, those of muscarine and nicotine, strongly developed, while the tetra-ethyl ammonium salts have them not at all. The intermediate compounds, with methyl and ethyl groups in varying proportions, show these actions in proportion to the number of methyl groups present. Again we are driven back on a conception of some peculiar conformation of the reactive surfaces to which the methyl groups are adjusted, which the ethyl groups will not fit.

A few years ago, in discussing this curious contrast between the methyl and ethyl ammonium bases, I ventured to say that it was as mysterious as the physiological contrast between sodium and potassium. Since that rash comparison was committed to print, the peculiar physiological position of potassium has been illuminated from an altogether unexpected quarter. Zwaardemaker has revealed its relation to the scarcely noticed fact that potassium is a weakly radioactive element. The key to the problem we have been discussing may not be found as suddenly, but I think it is not improbable that there are factors concerned in determining the activity of some of the organic compounds we have been considering, the importance of which is as little appreciated as was that of the radioactivity of potassium but a few years ago. This feeling, that we are always on the verge of some great clarification, is, I believe, a powerful element in the fascination which this problem of the relation between action and constitution has exercised on many minds. We have plenty of stimulating suggestions and seductive analogies—half-lights and elusive hints. Progress towards real clarity of conception can only come from the continuous co-operation and intercourse between the workers in all the different departments of scientific inquiry with which the problem makes contact. Particularly, I think, is it necessary to realize that the core of the problem is physiological. We have had big books on the subject, of great value as works of reference, written by chemists who were content to take their biological data at second or third hand, from sources of the most variable authority and who have extracted from such materials generalizations as to the function of particular groupings. We find, for example, a statement copied from one to another compilation of this kind, that nicotine and pilocarpine are closely similar in action, and that this is related to the presence in each of a five-membered ring. The real problem is created by the fact that the action of pilocarpine closely resembles, not that of nicotine, but that of muscarine and arecoline, and that no one hitherto has traced any structural or other similarity between these three; and that nicotine resembles in its action not pilocarpine, but lobeline and cytisine, which, again, have not been shown to have any other point of definite community with it. We need all the help that organic and physical chemistry can give, but the initiative and the guiding influence in the inquiry must come at least as much from the physiological side. Under such conditions I believe the quest is well worthy of an eager pursuit. It is not merely a question of finding a scientific basis for the production of more and better therapeutic agents than those which are yet available. There is a whole group of physiological and pathological problems here closely knit together, and, from whatever quarter the light comes, it cannot fail to illuminate the whole field.